INVESTIGATIONS INTO THE PILOT SCALE SEPARATION OF PROTEIN AND STARCH BIOPOLYMERS FROM OAT CEREAL.

A thesis submitted in fulfilment of the requirements for the Degree of

Doctor of Philosophy in Chemical and Process Engineering

in the University of Canterbury

by Rebecca J. Macdonald

University of Canterbury

2010

Acknowledgements

I have a great number of people to thank who have helped and supported me on this journey. Firstly and fore mostly I thank Ken Morison, my primary supervisor. I am grateful for the focus you brought to my work. You instinctively knew what support I needed at different times throughout this course of study; whether it was to listen, discuss or advise. I cannot thank you enough (or Annabel and Katie, your little helpers). Thanks also to my co-supervisors Conan Fee and Nigel Larsen. You were always happy to help, from assisting me with paperwork to providing specialist technical advice.

I am indebted to Rachael MacManus for her help with many of the experiments. You gave me ability to be in two places at once, by being available to switch on equipment, switch it off again later and take samples. Thanks also to Hillary Davidson, an engineering summer student. You helped establish some of the experimental protocols at the very beginning of this study. I also thank the rest of the AgResearch Bioprocess Engineering Team and the wider AgResearch Biobased Materials Team (especially Natasha Hammer, Paul Middlewood, Tess Engren, Matthew Paulik, Dr Sonya Scott, and Dr Alisa Roddick-Lanzilotta). You analysed many, many samples for me and gave me the time and space to carry out this study. Thank you Dr Charissa Cornellison and the AgResearch Proteomics Team (especially Dr Stefan Clarens and Dr Jeff Ploughman). Your specialist expertise in analytical proteomics was very helpful. Thank you Lidia Motoi from Plant and Food Research. You graciously gave me to access your specialist dough processing equipment and tested the protein content of many samples for me. Thank you Dr Ester Kim and Gail Krisnic. Your expert operation the confocal laser scanning microscope and scanning electron microscope was invaluable. Thanks you to Marco Morgenstern. You assistance and guidance with the dough rheology measurements was gratefully received. I also thank Dr Jafar Al-Hakkak and Fadia Al-Hakkak. You invented the Al-Hakkak Process, which was the focus of my studies. You kindly lent this process to me and gave the opportunity to carry out my engineering research.

I am grateful to my many sources of funding: the Biopolymer Network Limited for providing a platform for the overall research programme which was funded by the Foundation for Research Science and Technology, Technology New Zealand for awarding me an Enterprise Scholarship and Canesis Ltd (later AgResearch Ltd) generously providing the necessary co-funding for the Enterprise Scholarship and also providing me with the time and equipment to carry out my research. I am very grateful to Drs Dick and Mary Earle for awarding me the 2008 Earle Travel Fellowship. Your generosity enabled me to travel to Europe, meet other researchers working in similar fields and attend conferences and workshops relevant to my studies. Also Adze van der Goot and Elspeth van der Zalm from Wageningen University in The Netherlands. I really appreciate the short time I got to spend with you at your laboratory whilst I was the Earle Travel Fellow in 2008. Please stay in touch.

Thanks also to my family – you know who you are. You always knew I could and I did! Thank you William for creating a sense of urgency and giving me the timeline I needed to get the thesis written and submitted! Finally and most importantly my deepest, most heartfelt thanks go to my husband Greg Ellis. You gave me the confidence I needed to start, unwavering support during, and showed outstanding patience until I finished. Your proofreading efforts alone are worth a medal (oh, that's right, you already have one). Thank you Babe.

"Mātauranga"

(education, knowledge, wisdom, understanding, skill)

Abstract

Cereals contain naturally occurring biopolymers (for example proteins and starches) that can be used as renewable raw materials in a variety of speciality chemical applications. The separation of protein and starch biopolymers from wheat is well established and relies on a group of proteins called glutens that have a unique network-forming functionality. Oat and other cereals do not naturally contain these gluten proteins and typically rely on chemical-based separation techniques which alter the chemical and physical structures and damage the inherent natural functionality of the biopolymers.

This research study investigated the separation of the protein and starch fractions from cereals using the Al-Hakkak Process, a new aqueous process. This process involves adding water and wheat gluten protein to cereals that do not contain gluten. The wheat gluten interacts with the cereal proteins, facilitating the separation of the starch and protein fractions whilst retaining their inherent natural functionality.

The aim of this research project was to investigate and optimise the pilot scale separation performance of the Al-Hakkak Process using oat flour. As very little prior research had been carried out, the focus was to characterise the oat starch and protein separation performance and gain an understanding of the mechanisms involved. A variety of techniques were employed. Large scale deformation rheology was used to gain an understanding of the oat-gluten dough rheology and establish the relationship between the rheology and the separation performance. Confocal scanning laser microscopy was used to investigate the structure of the oat-gluten protein network. The molecular interactions between the oat and gluten proteins were studied using gel electrophoresis. The network-forming functionality of the new oat-gluten protein was explored. The influence of various processing parameters on the pilot scale separation performance was investigated and the results compared with other data collected through the study to identify key processing parameters. This research programme has resulted in interesting, encouraging and some unexpected outcomes and these are discussed in detail in the thesis.

It was concluded that an insoluble protein network formed in the oat-gluten dough and both kneading and extraction processes were found to contribute to the formation of this.

A key conclusion was that the changes that took place in the oat-gluten dough were similar to, but not identical to, the changes that occur in wheat dough. It was proposed that the mechanism for the development of a protein network in oat-gluten dough differed from wheat dough for two main reasons: a) the presence of the oat flour disrupted the normal wheat gluten behaviour, and b) components in the oat flour altered the activity of the gluten proteins. The research identified key processing parameters for the Al-Hakkak Process including kneading time, gluten content, and sodium chloride content of the oat-gluten dough as well as sodium chloride concentration, pH, and temperature of the extract liquor.

An important discovery was that the oat and gluten proteins interacted at a molecular level through reducible, covalent, bonding (most likely disulphide linkages) to form the insoluble protein network in the oat-gluten dough. It was concluded that these reducible bonds coupled the individual protein subunits to form new hybrid oat-gluten protein molecules (a combination of oat proteins and gluten proteins). Both insoluble and soluble proteins in the oat and gluten flour were involved in the formation of the insoluble protein network in the oat-gluten dough. This outcome has applications beyond the Al-Hakkak Process, as this new knowledge can be applied to the wider dough processing industry.

It was concluded that the wheat gluten was the source of the protein network-forming functionality of the hybrid oat-gluten protein and that the oat proteins had a diluting effect. It was proposed that oat-gluten protein flour from the Al-Hakkak Process could be reused to replace the commercial wheat gluten flour in subsequent production batches.

During spray drying of the starch stream, the soluble biopolymers in the extract liquor were found to act as an adhesive and glued individual starch granules together to form spherical agglomerates. Acidification of the extract liquor was found to enhance this agglomeration. It was proposed the acidified starch granules were sticker during spray drying due to the partial acid hydrolysis of the starch granule suface which enhanced the agglomeration.

Table of Contents

1. Introduction

1.1. Background

Cereals contain naturally occurring biopolymers (proteins, starches and lipids) which can be isolated and used as renewable raw materials in a variety of applications such as rheology modifiers and antioxidants. The characteristics of these biopolymers vary between different cereal varieties as does the relative amounts of the different biopolymers present. For example, oat contains starches with a uniquely small granule size as well as having particularly high levels of proteins containing the amino acid cysteine present. Some characteristics are considered more desirable than others (for example small granule starch) and can command a higher commercial value. Biopolymers with demonstrated functionality can have applications as ingredients in high value "niche" speciality chemicals products such as cosmetics.

The separation of the various polymer fractions of cereals is well established for wheat which contains proteins called glutens that facilitate this separation. However, the natural composition of many cereals such as oat that contain little or no gluten protein polymers makes it difficult to extract and separate the various polymer fractions whilst keeping the chemical and physical molecular structures intact. Chemical methods that are currently being used to carry out this separation typically solubilise one or more of the biopolymer fractions. This results in changes to the chemical structure of those biopolymer molecules, thereby altering their natural, inherent characteristics and functionality. It is desirable to maintain the natural characteristics and functionality of these biopolymers and research into separation processes that do not change the chemical composition of the biopolymers much needed.

1.2. Raw Material Selection

Proteins and starches can be obtained from a range of natural and renewable sources such as grains, legumes and root crops (for example potatoes). The characteristics of protein and starch vary, depending on the source, with some characteristics considered

more desirable than others. As a result the value of each biopolymer fraction varies as the characteristics vary.

Oat was selected as the candidate raw material for this research study. Oat is inexpensive and is typically grown for animal feed and to a lesser extent human consumption. Literature reports vary but in the 1990's about 83 % to 95 % of the worldwide production of oats went into low value animal feed products [1-3]. In 2007 oat had a value of US$181 per tonne compared to US$238 per tonne for wheat [4]. Oat grows well in cool, damp climates such as northern Europe, eastern Europe, northern USA, Canada and New Zealand [1, 5]. Compared to other cereals oat contains a number of interesting biopolymers and has unusual relative distribution of these biopolymers. Oat has higher levels of the amino acid cysteine than other cereals and a high oil content as well as being rich in beta-glucans (with reportedly cholesterol lowering activity) [1-3, 5]. Oat starch has a small granule size, averaging about 12 μm, compared with wheat starch with a typical granule size of 60 μm. In recent years, there has been an increase in the use of oats in non-food applications; oat flour has been used for its antioxidant and skin soothing properties in cosmetics, oat starch has been used in glues, and oat grain has also been used as a feedstock for furfural and furan production [1].

1.3. Purpose

The overall purpose of this research project was to investigate methods of separating the protein and starch fractions from cereals that contain little or no gluten. Oat was selected as the candidate raw material for this study. Processes were considered that had little or no effect on the chemical and morphological structure of the individual biopolymer molecules (for example, the physical conformation of the protein molecules), thereby maintaining the inherent natural functionality and characteristics of these naturally occurring biopolymers.

This research builds on the Al-Hakkak Process invented by Plant and Food Research Limited and now being further developed by the Biopolymer Network Limited. This

patented process involves the addition of gluten-containing protein flour to a non-wheat cereal flour such as oat flour. When water is added, the gluten proteins promote agglomeration of the proteins to form a relatively stable hybrid protein network, similar to wheat processing. At the start of this research project the process had only been carried out in the laboratory, with many of the processing steps only suited to very small scale, laboratory techniques. Significant research was required to understand the mechanisms involved and create industrially scalable processes, robust enough to absorb variations in raw material composition, whilst also producing a uniform product.

1.4. Objectives

The overall objective of this research was to create and test pilot scale processes for the separation of the protein and starch fractions from oat by applying and fine tuning the Al-Hakkak Process technology. A key factor for success was maintaining the natural functionality of the protein and starch fractions. Oat was selected as the candidate cereal as it contains biopolymers with interesting natural functionality (for example starch with a small granule size and proteins rich in cysteine).

The specific objectives of the research project were:

- Investigate the importance of oat-gluten dough rheology on the separation of protein and starch using the Al-Hakkak Process.
- Understand the structure and functionality of the oat-gluten protein network formed in the Al-Hakkak Process and relate this to the separation efficiency of protein and starch.
- Investigate the effect of variable oat-gluten dough composition on the performance of the Al-Hakkak Process
- Investigate the effect of varying processing conditions on the performance of the Al-Hakkak Process
- Establish and test the Al-Hakkak Process at pilot scale using commercially available equipment.

1.5.Challenges

This research project had two distinct elements that were interrelated.

Firstly, challenges that must be overcome associated with raw material variability. Naturally occurring raw materials can vary in composition depending on a range of factors, such as the growing conditions of the plant, geographical location, grain storage conditions, and seasonal variation. The development of a robust pilot scale Al-Hakkak Process required gaining an understanding of the impact of these variations.

The second challenge that must be overcome was associated with the processing required to generate the final product. The final product from the Al-Hakkak process must be consistent and uniform regardless of variation in the raw material and be in the desired form for commercial sale. The pilot scale Al-Hakkak Process needed to have processing flexibility to manage these natural variations.

This research project is the first comprehensive and systematic analysis of the Al-Hakkak Process. It was needed to gain an understanding of the key mechanisms underlying the individual unit processes and create a robust platform for future research and commercialisation of the process.

1.6.Limitations

This research project was sponsored by the Biopolymer Network Limited (BPN), a collaboration of three research organisations (Scion, Plant & Food Research and AgResearch Limited) focused on the development of bio-derived chemicals.

This research project formed part of a FRST funded research programme conducted by the BPN, titled “Biopolymer Products”, Contract Number BPLY0401. The aims, objectives and outputs of this research project must be consistent with overall research programme. This research project forms part of Objective 1 - Manufacture of Core Chemical Entities, which has the following overall objective statement:

> *"The goal of this objective is to deliver viable turn-key manufacturing processes for the isolation of core chemical species from key components of the New Zealand bioresource. This objective contributes to the programme outcome by providing well characterised biological extracts that form the basis of new export products, and creates and secures IP related to their production. This objective further contributes by capturing global knowledge and applying it to the New Zealand resource, for the purpose of creating new business opportunities. Close interaction with industry through Objective 4 will guide developments in this objective. The outputs of this objective will provide novel biological extracts for Objectives 2 and 3 to react and formulate from year 2 onwards."*

1.7.Organisation of the Thesis

This thesis is presented in several chapters:

1. Background (this section)
2. Literature Review
3. General Methods
4. Oat Starch Drying
5. Oat-Gluten Dough Rheology
6. Oat-Gluten Protein Structure
7. Oat-Gluten Protein Interactions
8. Oat-Gluten Protein Functionality
9. Al-Hakkak Process Variability
10. General Discussion and Summary

Chapter 2 comprises the overall literature review and describes the literature landscape. It includes a broad review cereal composition (including oat) and existing processes for separating cereal proteins and starches. Several experimental methods were common to several sections of the research study and these general

methodologies are described in Chapter 3. Chapters 4 to 8 summarise the technical investigations that were undertaken. Each of these chapters includes a literature review relevant to the technical content of that chapter. These chapters each contain sections on methodology, experimental results, discussion and conclusions. Chapter 9 describes the process variability investigations and scale up considerations. It draws on some of the results and conclusions from earlier in the study and applies these to the Al-Hakkak Process. The final chapter discusses and summarises the key research outcomes from this study.

2. Literature Review

2.1.Introduction - General

This chapter comprises a broad, overarching literature review. It provides a summary of the current knowledge of oat composition and processing. It includes a detailed summary of the composition of oats and other cereals, particularly wheat. Current techniques for processing oats to separate protein and starch are reviewed. Processes for extracting biopolymers from wheat are also discussed, where they are considered relevant to this study. Subsequent chapters discussing the technical investigations undertaken in this study also include smaller, but more focused literature reviews. These reviews are specific to the technical content of each chapter.

This broad literature review is written in two sections: 1) Cereal Composition and 2) Processing.

2.2.Introduction - Cereal Composition

The following sections of Chapter 2 provide an overview of the composition of cereals focusing on oat and comparing oat to wheat.

Wheat is considerably better documented in literature than oat. The characteristics of oat compared to wheat are described in this chapter, including the variability of oat composition that has been well reported in literature. The unique characteristics of the gluten proteins contained in wheat and how these provide a mechanism for the separation of protein and starch is discussed. Only the composition of processed oat products such as flour and groats (the whole grain) has been considered. Other parts of the oat, such as leaves, stalk and husk are beyond the scope of this study and are not considered.

There is a wealth of information published on the composition of cereals, particularly wheat, and this literature review identified five recent text books that give a good

summary of the current knowledge. Much the information presented in this chapter on cereal composition is summarised from these texts.

- Modern Cereal Science and Technology [6]
- Cereal Grain Quality [7]
- Wheat Production and Utilisation [8]
- Wheat Production, Properties and Quality [9]
- Fermented Cereals, A Global Perspective [2]

Compared to wheat, there is a lot less information published on the composition of oat. This literature review identified four recent text books containing useful general information on the composition of oat (flour and groat). Much the information presented in this chapter on oat composition is summarised from these texts.

- The Oat Crop – Production and Utilisation [10]
- Oat Science and Technology [3]
- Oats: Chemistry and Technology [5]
- Oats and Oat Improvement [11]

2.3. Background

2.3.1. Cereals

Cereals are members of the Graminea (grasses) and have a similar structure with common distinguishing features, such as being monocotyledons (single cotyledon/seed leaf). Cereal grain (botanically referred to as the caryopsis) is the seed and consists of pericarp (fruit coat), testa (seed coats) and the seed itself. The bulk of the seed is taken up by the endosperm (food reservoir) with the germ accounting for only a small fraction of the seed (see Figure 2.1). The pericarp and testa, along with the nuclear tissue and aluerone cells make up the bran fraction. The endosperm consists of two cell types: 1) starchy endosperm, containing the starch granules embedded in a protein

matrix, and 2) aleurone, one or more layers of cells that cover the endosperm. The germ (including the embryo and scutellum layer) is located within the endosperm.

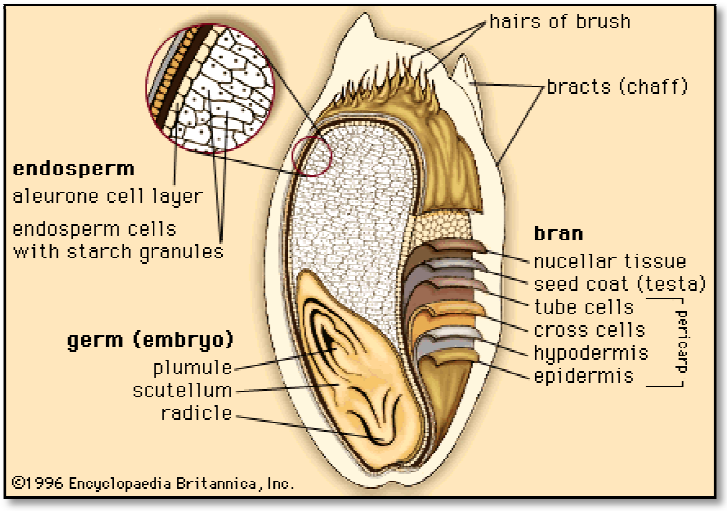


Figure 2.1. Diagrammatic illustration of wheat grain [2].

Cereals contain high levels of carbohydrates (for example starch, dextrins, pentosans, and sugars) and low levels of proteins and lipids. The distribution of the individual chemical components varies with the individual structural components that make up the grain. Wheat grain is well studied and the structure and composition of wheat is well documented in literature. Table 2.1 summarises the contribution of different parts of the wheat grain to the total mass [6]

Table 2.1: Wheat Grain Composition [6]

	Protein	**Fat**	**Fibre**	**Ash**	**Starch***
Pericarp	5	1	21	3	70
Aleurone	18	9	7	16	50
Endosperm	10	1	>0.5	0.5	88
Germ	26	10	3	5	56

* by difference

2.3.2. Oat

Oat is a cereal and a member of the genus *Avena*, comprising a variety of cultivated species [12]. Oat is classified in to three main species, *Avena sativa* (white or yellow oats), *Avena byzantina* (red oats), and *Avena nuda* (naked oats) [12]. However, the majority (over 75 %) of commercially grown oat is of a single species, *Avena sativa*, and as a result this is the focus of most of the information in literature [12, 13]. This study considers commercial oat of the *Avena sativa* species. Within the *Avena sativa* species, there are many certified cultivars with differing composition. Each cultivar is described by twenty-six plant characteristics, each having two to five states. Of these, four characteristics are widely used for distinguishing between the different cultivars: hairiness, glaucosity (waxiness) of the lemma, colour of the lemma, and seasonality [12].

The structure of all oat grain species is similar to other cereals and contains three main layers: the bran, the endosperm and the germ (see Figure 2.2) [2, 3, 5, 10, 11]. Each of these layers contains several sub layers. The outer layer (bran) contains the pericarp, testa, and nuclear tissue. These structures are fibrous and contain mainly complex carbohydrates along with lignins and other phenolic compounds. In oat, the seed is enveloped in a hull comprising of floral envelopes, which typically accounts for 30 % of the mass of the grain. The aluerone layer of oats consists of a single layer of cells separating the endosperm from the bran and is higher in fat and protein than the endosperm. The cell walls are rich in beta-glucan and a range of phenolic compounds. The cell interior contains protein bodies and lipid droplets. The endosperm is the main fraction of the oat grain, containing between 55 % to 80 % of the total mass. The endosperm contains cells that consist of starch granules and spherical protein bodies. Oat differs from other cereal in that the endosperm contains a signification amount of beta-glucan (typically 2.2 % to 6.6 %), originating from the endosperm cell walls. The endosperm also has high lipid content, up to 8 %, compared with typically up to 1.0 % for wheat. The germ includes the embryo and scutellum layer and is a minor fraction of the oat grain, accounting for less than 4 % of the mass. The germ contains higher levels of lipid and protein, compared with the endosperm.

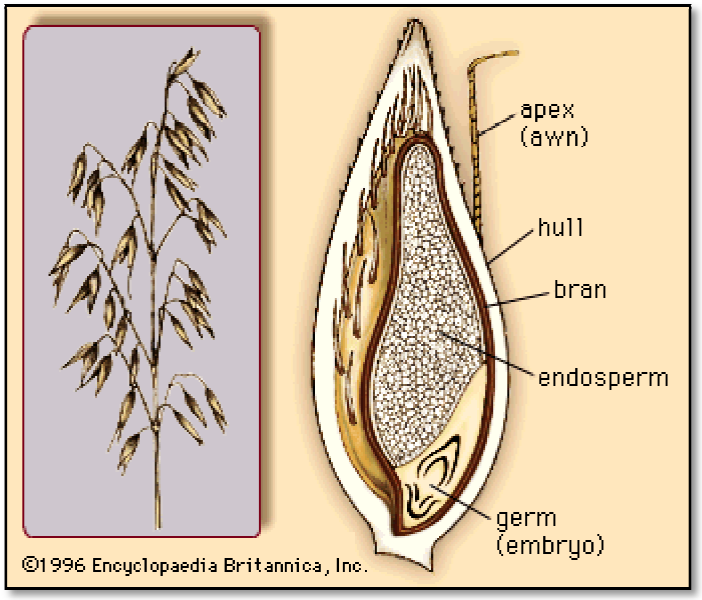


Figure 2.2. Diagrammatic illustration of oat grain [2].

2.4.Composition Variability

Cereals including oat are a naturally occurring biobased material, and, as such, their composition displays natural variation. The naturally occurring variation in the composition of the oat grain (*Avena sativa*) has been well summarised by several authors [12, 14, 15]. These authors show that variations are due to a range of factors including cultivar, environmental factors and processing.

Oat is generally purchased as a bulk commodity product consisting of a blend of oat grains from a range of varieties. Hence, the composition extremes are lessened by the blending processes [12]. Table 2.2 shows the typical composition of commercially available oat groat blend in the USA and the typical range of variation [12].

Table 2.2: USA Oat Composition Variability [12]

Component	Average value (%)	Typical range (%)
Proteins	15.2	11 – 20
Fats/lipids	7.6	5 – 9
Starch	51.1	44 – 61
Beta-glucans	4.2	2.2 – 6.6
Ash	1.9	1.3 – 2.3
Free sugars	8.9	7.0 – 11.0
Dietary fibre	1.1	0.9 – 1.3
Moisture	10.0	9 – 14

Variation in the reported composition can result from environmental factors (such as climate, soil type, season, geographical location etc) as shown in Table 2.3 [14, 16, 17]. Literature reports that the variation in the oat crop is due primarily to environmental factors [14, 15].

Table 2.3: Oat Composition – Seasonal Environmental Factors [16]

Component	Seasonal range (%)
Starch	54.6 – 64.0
Crude protein	15.3 – 18.9
Fats/lipids	5.73 – 6.86
Ash	1.60 – 2.26

Composition also varies with cultivar and there are 127 different oat cultivars listed on the EU Common Catalogue [12, 13, 16, 17]. Table 2.4 summarises the reported variation in composition of oat due to cultivar.

Table 2.4: Oat Composition – Cultivar Variations [16]

Component	Range among cultivars (%)
Starch	57.1 – 60.4
Crude protein	14.6 – 19.6
Fats/lipids	4.64 – 7.81
Ash	1.84 – 2.13

The distribution of the individual chemical components varies with the individual structural components that make up the grain. Literature reports that variation in composition can result from prior processing or treatment, such as storage conditions, dehulling (groat/kernel), rolling (rolled oats), and milling/grinding (meal or flour), due to the components being removed during each process step [14, 18]. The chemical variation of the individual structural components of wheat grain was summarised previously in Table 2.1 [6]. Table 2.5 summarises the variation in composition of oat from a single source depending on the prior treatment.

Table 2.5: Oat Composition – Processing Factor Influence [14]

Component	**Dry milled oat** (%)	**Finished groats** (%)	**Oat flour/chips and meal** (%)
Moisture	7.0	7.0	7.5
Crude protein	12.1	15.8	15.5
Crude fat	5.1	7.2	6.2
Crude fibre	11.0	1.5	3.6
Ash	3.4	1.9	2.1

Variations in composition arising from the different analytical methods used to identify the individual components have been noted in literature [14]. Table 2.6 summarises the variation in composition of various oat products from different literature sources.

Table 2.6: Oat Composition – Different Analytical Methods

Component	**Whole oat** (%) [14, 18]	**Oat grain (**%) [2]	**Rolled oat** (%) [14, 19]	**Oat flour (**%) [18]
Moisture	9.9	-	8.2 – 11.5	6.4 – 6.9
Crude protein	9.8 – 12.9	9.3	11.2 – 17.0	11.8 – 27.5
Lipid/oil	4.1 – 5.7	5.9	4.8 – 9.2	5.2 – 8.5
Fibre	10.7 – 13.0	2.3	1.1 – 1.3	0.6 – 2.8
Ash	3..0 – 3.5	2.3	1.3 – 1.9	0.7 – 4.1
Carbohydrate	59.4 – 68.9	62.9	65.0 – 72.8	74.8 – 50.3

2.5.Chemical Composition

The composition of cereals, particularly wheat, is well documented in literature and discussed in detail in the standard texts listed at the start of the chapter. Cereals are normally reported as comprising six main component categories: water, crude protein, lipid/oil, fibre, ash and nitrogen free extracts (carbohydrate) [1, 12, 14]. Compared to other cereals oats typically have higher protein and fat contents and lower carbohydrate content, but the carbohydrate is still the major constituent [20].

2.5.1. Carbohydrates

For all cereals, including oat, carbohydrates constitute the largest component generally contributing over 60 % of the total dry mass of the cereal grain [2, 6, 14]. Carbohydrate is a broad description of a wide variety of molecules which includes simple sugars, starch (sugar polymers), and non-starch polysaccharides [2, 6, 14]. All are present in cereals.

Carbohydrates can be grouped by a variety of factors such as solubility, digestibility, starch and non-starch polysaccharides. Literature generally reports two broad component categories: starch and non-starch polysaccharides which includes soluble simple sugars as well as fibre (including soluble polysaccharides, insoluble polysaccharides and lignins) [14, 21]. Values for the carbohydrate and the various carbohydrate fractions reported in literature vary, due to the different analytical techniques used, with some calculated ‘by difference’ [14].

Compared to the other cereals, there is little information published on the chemical and functional properties of oat and some of the information that is published is contradictory [22, 23].

2.5.2. Starch

Starch is the principle carbohydrate in cereals, including oat, and is the primary storage component found in the endosperm of grain [16, 21, 24]. Starch accounts for the majority of the mass of the grain and generally accounts for up to 80 % of the total

carbohydrate present, depending on the cereal of interest [2, 14]. Literature reports vary and some authors report that starch comprises up to 73 % total carbohydrate present in the oat grain [14, 17]. Table 2.7 shows the variation of the contribution of starch to the total mass of oat reported in recent literature [14, 21].

Table 2.7: Starch Content of Oat

Oat Groat (%) [14]	**Oatmeal** (%) [14]	**Oat Groat** (%) [21]	**Milled Oat** (%) [21]
49.0 – 75.2	64.3 – 72.6	43.7 – 61.0	67.0 – 73.5

Welch RW (1995) [14]
Peterson D (1992) [21]

Molecular Composition of Starch

Starch is a naturally occurring biopolymer and comprises of two separate main fractions: amylose and amylopectin [8, 14, 23, 25, 26]. Both these fractions are high molecular mass polymers [20].

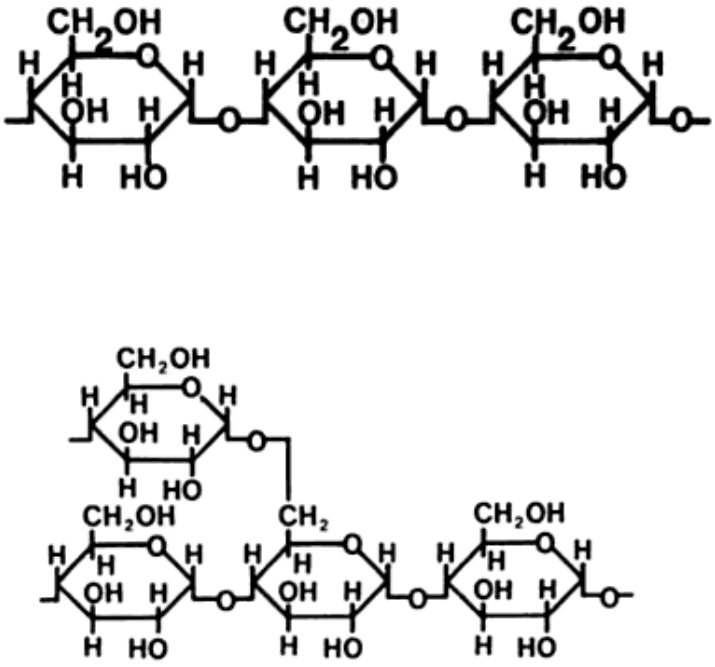


Figure 2.3. The typical structure of the (A) amylose and (B) amylopectin polymers [20].

Amylose is a straight chain polymer of glucose, consisting of end to end α(1→4) linkages and less than 1 % branching α(1→6) linkages (see Figure 2.3) [8, 14, 18, 20, 24-26]. Literature reports that amylose has a molecular mass of 10^5 to 10^6 Da [24, 25]. The degree of polymerisation of the individual molecules is reported as 1500 – 6300 glucose units for wheat starch [24]. Amylose has high solubility in hot water [24].

Amylopectin is a glucose polymer with a branched structure comprising of both straight chain α(1→4) and branched α(1→6) linkages (see Figure 2.3) [8, 14, 18, 20, 25, 26]. A molecular mass of $>10^8$ Da is reported in literature for amylopectin [24, 25]. Branching occurs every 20 to 25 glucose units (5 %), depending on the cereal, and the degree of polymerisation of the individual molecules are reported as 10^4 to 10^7 glucose units for wheat starch [24]. Amylopectin has lower solubility than amylase in hot water [24].

The reported composition of oat starch varies [14, 21, 23, 27]. Table 2.8 summarises the main molecular fractions of oat starch (amylose and amylopectin) reported in recent literature.

Table 2.8: Oat Starch Mass Fractions

Starch Fraction	**Mass fraction**			
	(%) [28]	(%) [14]	(%) [21]	(%) [23]
Total amylose	22.1 – 26.6	17.5 – 33.6	25.2 – 29.4	27.5 – 29.8
Apparent amylose	-	-	-	19.7 – 22.0
Protein	-	0.3 – 0.95	-	-
Lipid	1.08 – 1.18	0.7 – 2.5	0.67 – 0.11*	0.66 – 0.75
			1.35 – 1.52 *	

Wang, L and White, P (1994) [28]
Welch, RW (1995) [14]
Peterson, D (1992) [21]
Tester, R and Karkalas, J (1996) [23]
*using different extraction methods

The degree of polymerisation of the amylose fraction of oat starch has been measured as 939 to 1208 (mass average) and 392 to 2920 (apparent) glucose units [27]. The

degree of polymerisation of the amylopectin fraction of oat starch has be measured as 17 to 204 (weight average) glucose units [27].

Cereal starch contains low amounts of lipids, including tricylglycerides, diacylglycolipids and phosphopilids [29]. Cereal starch has been found to contain various proteins with some associated with the starch granule surface (surface proteins) and others contained within the starch granule structure (integral proteins) [26].

Oat starch contains minor amounts of both protein and lipid [14, 21, 23, 30]. Oat starch has higher levels of lipids than other cereals and these lipids comprise primarily of lysophospholipid and free fatty acids [14, 21, 28, 30].

Starch Granules

Cereal starch is naturally present in the grain as insoluble particles usually termed "granules". The size and shape of the granule varies between different cereals [20, 24-26, 31]. Oat starch granules exist as both single granules and agglomerated clusters of granules usually termed "compound-granules" [21, 23, 24].

Individual oat starch granules are smaller than the granules from other common cereals, such as wheat [17]. Literature reports that typically the individual oat starch granules are polyhedral in shape and range in size from 3 μm to 12 μm. In oat compound-granules have a typical size range of 60 μm to 150 μm [20, 23, 24, 26, 30, 32, 33]. Reported variations between oat cultivars for individual starch granules range from a mean diameter 4.96 μm to 5.63 μm and the mean volume 94 μm^3 to 146 μm^3 [6, 21, 23]. The measurement technique used can influence the reported measured granule size [32].

Starch Functionality

This literature review has found that little research has been published on the functionality of the starch component of oat, with investigations into the oat carbohydrates focused on the non-starch components such as the soluble sugars and the fibre component, in particular the beta-glucan [16, 22, 23]. Oat starch granules,

like all cereal starch granules, are insoluble in cold water, but absorb some water (0.4 g water per 1 g starch) [34]. On heating starch granules swell and irreversibly solubilise to form a paste [20, 24, 26]. This process is termed “gelatinisation”. The solubility of oat starch increases as temperature increases, reported as 4.1 % to 6.0 % at 85 °C increasing to 33.5 % to 43.3 % at 95 °C [28]. Swelling power is a measure of the amount of water being absorbed into the starch granule and is often used to characterise the changes in the starch granule during heating and gelatinisation. Table 2.9 shows the reported effects on swelling power of oat starch granules.

Table 2.9: Oat Starch Granule Swelling

	80 °C	**85 °C**	**95 °C**
Swelling power [28]	-	8.7 % - 9.6 %	27.8 % - 34.8 %
Swelling power [33]	-	-	22.4 % - 27.4 %
Swelling power [23]	8.6 % - 10.0 %	-	-

Wang, L and White, P (1994) [28]
Hoover, R and Vasanthan, T (1992) [33]
Tester, R and Karkalas, J (1996) [23]

The gelatinisation of oat starch is reported in literature as varying with cultivar, with variations reported in the gelatinisation onset temperature, gelatinisation peak temperature, and gelatinisation conclusion temperature as well as the swelling power [22, 23, 26]. Literature reports that the gelatinisation properties of oat starch differs from other cereal starches with oat having a comparatively lower gelatinisation temperature [20, 22, 28, 30]. Table 2.10 summarises the gelatinisation characteristics of oat starch reported in a comprehensive study by Tester and Karkalas [23].

Table 2.10: Oat Starch Gelatinisation Characteristics [23]

Gelatinisation parameter	**Temperature**
Onset temperature (T_O)	44.7 °C – 47.3 °C
Peak temperature (T_P)	56.2 °C – 59.5 °C
Conclusion temperature (T_C)	72.0 °C – 73.7 °C

Like many cereal starches, oat starch shows B-type viscosity profile [35]. B-type viscosity profile displays a lower peak and less thinning during cooking compared to

A-type starches. Starch granules with a B-type viscosity profile do not swell as much and are less susceptible to damage. However, x-ray diffraction of oat starch shows an A-type pattern [33]. Literature reports that oat starch has an unusually high viscosity on cooling, with the resultant cooled gel being clearer, less firm, more elastic, more adhesive and less susceptible to degradation than cooled starch gels from other cereals such as wheat [6, 20-22, 24].

2.5.3. Soluble Sugars

The soluble sugars are a minor component of the oat and their concentration in oat flour is low compared to many other cereals reported in literature [17, 21]. This soluble fraction is reported in literature to include a range of monosaccharides and disaccharides as well as some oligosaccharides [14]. Table 2.11 summarises the soluble oat sugars reported in literature.

Table 2.11: Key Soluble Sugar Fractions in Oat

Sugar fraction	**Oat flour** (g/kg dry) [14]	**Oat grain** (g/kg dry) [14]	**Oat flour** (g/kg dry) [21]
Total sugars	6.0	11.2	9 – 13
Fructose	0.3	0.4	0.2 – 0.5
Glucose	0.6	0.7	0.6 – 0.7
Maltose	0.2	-	0.1 – 0.3
Sucrose	4.9	10.1	4.0 – 6.3
Raffinose	2.1	1.5	1.6 – 2.6
Stachyose	0.8	-	0.7 – 0.8

Welch RW (1995) [14]
Petersen, D (1992) [21]

2.5.4. Non-Starch Polysaccharides

The non-starch polysaccharides component (often referred to as “NSP” or “crude fibre”) of the carbohydrate fraction comprises a diverse range of water soluble and water insoluble molecules generally referred to as “soluble fibre” and “insoluble fibre” [14, 21, 36]. The primary molecular constituents of the non-starch polysaccharides include: lignin, pectin, cellulose, and hemicellulose (including pentosans) [2, 21]. These can be further split into water soluble non-starch polysaccharides (including

some beta-glucans and arabinoxylan) and water insoluble non-starch polysaccharides (including some beta-glucans and lignins) [36].

The total non-starch polysaccharides of oat is reported as being 10.2 % to 12.1 % [21, 36]. Reported values for the composition of the non-starch polysaccharides of oat vary widely between different samples and the measurement technique used. Cultivar and environmental factors are reported to influence the content of non-starch polysaccharides of oats [14, 36]. Table 2.12 summarises the fibre content of oats reported in recent literature.

Table 2.12: Non-Starch Polysaccharide Fractions Oat (dry basis)

Processing	**Total NSP** (%)	**Soluble NSP** (%)	**Insoluble NSP** (%)
Oatmeal [14]	7.6 – 12.1	4.0 – 4.9	3.2 – 7.2
Rolled oats [14]	9.9 – 10.5	4.2 – 5.4	5.1 – 5.7
Oat groat [14]	9 – 11	3 – 3.5	6 – 8
Milled oats [36]	10.2 – 12.0	4.1 – 4.9	6.0 – 7.1

Water soluble, non-starch polysaccharides includes some beta-glucans and arabinoxylan and insoluble non-starch polysaccharides includes other beta-glucans and lignin [36]. Breakdown of the soluble non-starch polysaccharides into total neutral sugar fractions shows that the soluble fraction consists mainly of glucose with intermediate amounts of arabinose, galactose and xylose and only small amounts of mannose and ribose. The insoluble fraction consists of arabinose, glucose and xylose with small amount of galactose, mannose and ribose [36]. The beta-glucan content accounts for the glucose present in both the soluble and insoluble non-starch polysaccharides fractions [36]. Table 2.13 summarises the reported variation of the main components of the soluble and insoluble, non-starch polysaccharides of oatmeal.

Table 2.13: Main Non-Starch Polysaccharides Fractions of Oatmeal (dry basis)

	Total neutral sugars (%)	**Beta-glucans** (%)	**Uronic acids** (%)	**Klason lignin** (%)
Soluble [36]	4.0 – 4.8	3.2 – 3.9	0.1	-
Insoluble [36]	2.8 – 3.8	1.2 – 1.7	0.4 – 0.5	2.6 – 2.8
Soluble [21]	0.8	3.6	0.1	-
Insoluble [21]	3.1	0.6	0.3	3.3

The main fraction of the soluble non-starch polysaccharides is beta-glucan, a linear chain polymer, consisting of D-glycopyranosyl units, irregularly configured with beta-1→3 and beta-1→4 linkages [14, 17, 21]. Beta-glucan is well reported in literature, probably due to the reported cholesterol lowering activity and other reported physiochemical functionality [14, 21]. Beta-glucan produces highly viscous solutions in water. Some authors report that not all of the beta-glucan is readily soluble [14, 21]. The beta-glucan concentration in oat is variable and Table 2.14 summarises the beta-glucan content of oats reported in literature [14, 16, 21, 37].

Table 2.14: Beta-Glucan Content of Oat (dry basis)

Processing	**Mean**	**Reported range** (%)
Groat [37]	1.0 – 5.0 [a]	-
Groat	2.3 – 5.1	1.8 – 7.5
Groat [14, 21]	2.5 – 4.2	2.7 – 6.3 [b]
Grain [14]	3.2 – 3.6	2.2 – 4.5
Grain [21]	1.0 – 5.6	-
Rolled oats [21]	-	4.3 – 4.6 [c]

a) Results of glass house grown oat species.
b) Not all data sets reported a range.
c) 41 % to 57 % of this was reported as soluble.

Literature reports low concentrations of the pentosan arabinoxylan in both the soluble and insoluble fractions, although the presence of arabinoxylan in the soluble fraction has been assumed from the presence of it breakdown products (arabinose and xylose) [14, 17, 21]. Little data is reported on the actual concentration of pentosans present in oat, although one reference reported pentosans concentrations of 2.1 % to 2.2 % in oatmeal and 2.4 % to 4.5 % in groat [14].

2.5.5. Proteins

Total Protein

Cereals contain different amounts of total protein and Table 2.15 shows the typical total protein of some common cereals [6]. The protein content and protein composition of oat differs from that of other cereals with the protein content of oats higher than most other cereals [17, 21]. Typical protein content of oat groats is shown Tables 2.16 and 2.17.

Table 2.15: Typical Protein Content [6]

Cereal	**Protein content** (%)
Oat	8 – 20
Wheat	10 – 15
Durum wheat	12 – 16
Barley	10 – 16
Rye	9 – 14

Table 2.16: Protein Content of Oat Groats

	Oat samples in range	**Mean** (g/kg)	**Reported range** (g/kg)
Oat groat [38]	289	171	244 – 124
Oat groat [39]	11 [b]	271	178 – 371
Oat groat [21]	50 [a]	160 - 174	145 – 197

a) 2 locations over three growing seasons.
b) 11 different species.

Table 2.17: Protein Content of Oat Commercially Milled Oat [39]

Milled oat fraction	**Reported range** (%)
Heavy oats	13.4
Light oats	9.6
Groat	18.9
Hulls	5.7
Flakes	17.6

Protein Fractions

Cereal proteins contain a variety of different types of protein molecules. These are commonly characterised by two methods: amino acid profile, and solubility characteristics as summarised in Figure 2.4.

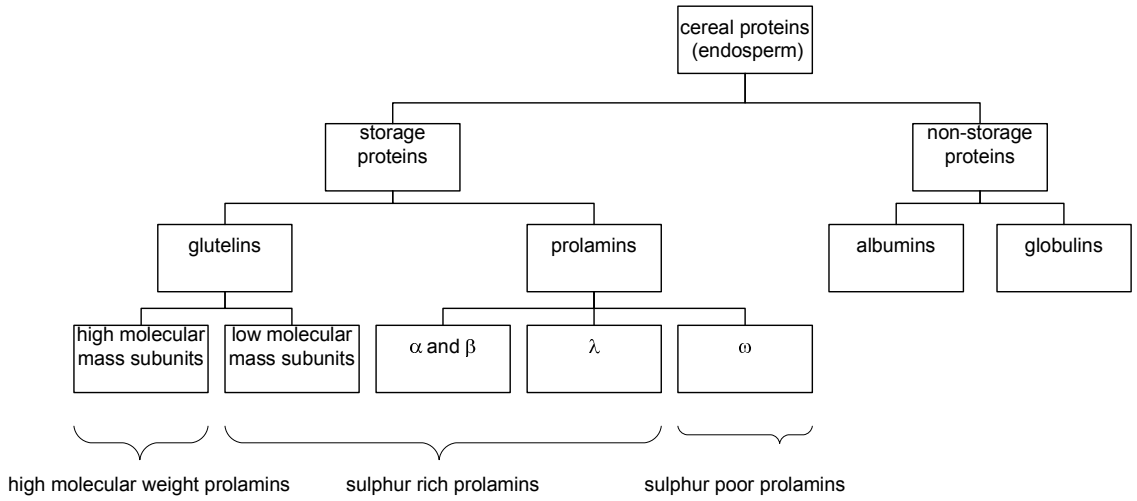


Figure 2.4. The major endosperm proteins from cereals (adapted from Shewry *et al.* (1986)) [41].

The solubility fractions of cereal proteins were first characterised by Osborne in 1924 and are most simply described as follows: [6, 14, 40, 41]

- Albumins – water soluble proteins.
- Globulins – proteins soluble in dilute aqueous salt solutions.
- Prolamins – proteins soluble in aqueous alcohol (termed gliadin in wheat and avenin in oat)
- Glutelins – proteins soluble in dilute acid or alkali (termed glutenin in wheat and oat).

The proteins are not evenly distributed throughout the grain; the endosperm proteins consist mainly of prolamins and glutelins (storage proteins), the embryo proteins comprise mainly of albumins, globulins and proteases (non-storage proteins) and the

bran proteins are mainly prolamins [6]. In all cereals the endosperm contains most of the protein [41].

The typical contribution of each protein solubility class varies between cereals and between cultivars [6]. As shown in Tables 2.18 and 2.19, the primary storage protein of oat is the globulin fraction, unlike other cereals, where the primary protein is the prolamin (avenin) fraction [17, 21, 42].

Table 2.18: Distribution of Cereal Proteins by Solubility Class [6]

Cereal	**Albumins** (%)	**Globulins** (%)	**Prolamins** (%)	**Glutelins** (%)
Oat	5 – 10	50 – 60	10 – 15	5
Wheat	10 – 15	5 – 10	40 – 50	30 – 40
Durum wheat	12 – 16	5 – 10	40 – 50	30 – 40
Barley	10 – 16	10 – 20	35 – 45	35 – 45
Rye	9 – 14	5 – 10	20 – 30	30 - 40

Table 2.19: Typical Oat Protein Fractions [14]

Protein fraction	**Typical range**
	% of total protein
Albulmins	14.4 – 20.1
Globulins	47.1 – 53.2
Prolamins	7.2 – 9.9
Glutelins	21.4 – 26.7

Differences in analysis techniques have resulted in differences in reported solubility fractions for oat, particularly the globulin fraction and this is discussed later.

Wheat glutenins are polymeric with a molecular mass of 3,000,000 Da, with high molecular mass subunits ranging in size from 80,000 Da to 120,000 Da and low molecular mass subunits (40,000 Da to 55,000 Da) [34, 43]. In wheat glutenin the ratio of low to high molecular mass subunits is reported as at least 2:1. The wheat glutenins contain both intra- and inter-molecular disulphide bonds. Inter-molecular bonding is reported to occur at the cysteine residues located at both ends of the high molecular mass subunits and one end of the low molecular mass subunits. Wheat gliadins are single chain, monomeric proteins with a relatively low molecular mass

distribution, typically 30,000 Da to 100,000 Da. Intra-molecular disulphide bonds occur, but inter-molecular bond do not form (i.e. gliadin protein molecules do not link with other proteins to form polymers).

Oat proteins are less well studied than wheat proteins. Oat glutelins are polymeric with a molecular mass of 322,000 Da, with large subunits ranging in size from 32,500 Da to 40,000 Da and small subunits ranging in size from 20,000 Da to 25,000 Da [21, 40, 42]. The pI is reported as 5.9 to 7.2 and 8.7 to 9.2 for the large and small subunits respectively [21]. Data suggests that the native protein is a hexamer of di-sulphide linked large and small subunits. [21, 40]. Oat prolamins (avenins) are reported as heterogeneous with a molecular mass of 22,000 Da to 43,000 Da [21, 40]. The avenins are reported to vary between oat species [21].

Differences in analysis techniques have resulted in differences in reported solubility fractions for oat, particularly the globulin fraction [40, 42]. The standard Osborne Solubility method does not accurately identify the globulin fraction in oat and recent research indicates that that some globulins have been incorrectly identified as glutelins [14, 40, 42]. To accurately identify the globulin fraction requires complete solubilisation of the proteins [40]. Recent literature suggests that the glutelin content may be lower than 5 % to 10 % of the total oat protein [40].

Differences in analysis techniques have resulted in differences in reported amino acid composition of oat proteins, particularly in earlier data [14, 44]. The amino acid profile for oat has been reported in literature [14, 39, 40, 45]. Published data from different regions shows broadly similar amino acid profiles for oat groats [14]. Environmental factors, such as soil nitrogen levels have been shown to affect the amino acid profile of oat with an increase in soil nitrogen resulting in increases in the grain yield and total nitrogen content of the grain [14, 17].

The four different Osborne Solubility Fractions have different amino acid profiles [14, 40]. The breakdown amino acid profiles for the four solubility fractions have been reported in literature for oat [6, 14, 40]. Higher total protein content of oat seems to be primarily related to higher content of the globulin fraction [44]. The amino acid

composition of oat is similar to the globulin fraction, and the overall amino acid profile of oat changes little with changing total protein content [44].

Other protein fractions exist in cereals (including oat) in very small amounts, including hydrolytic enzymes (used to hydrolyse the storage reserves, particularly in barley), hydrolytic enzymes inhibitors, and starch granule proteins (less than 0.2 % of the total protein) [7-9].

2.5.6. Lipids

Lipids are defined as the fat or oil components of cereals, extractable by a defined range of organic solvents [14]. Cereal lipids include triglycerides, phospholipids, glycolipids, free fatty acids, partial glycerides, sterols, and sterol esters [21]. The majority of lipids in wheat are the fatty acid esters of glycerol [29]. Other lipids include free fatty acids, several sterol based lipids and glycosphingolipids (including the triglycerides) [29].

Most of the lipids in wheat and other cereals are stored as oil droplets in the scutellum and alerone, but some lipids are present in the starchy endosperm [29]. The amount of lipids and the distribution of lipids throughout the grain varies with environmental and cultivar differences [29]. Differences in the sampling methods and extraction techniques have lead to differences in the reported lipids composition of the different cereals [21]. During milling the physical action can transfer some of the scutellum and alerone lipids into the starchy endosperm and resulting flour product [29].

Oat has a high lipid content compared to other cereals [1, 17, 30, 46, 47]. Whilst the lipid content of oat varies, most cultivars have a lipids content of 4 % to 6 % of the grain, although some literature sources have reported as low as 2 % and as high as 8 % [14]. The lipid content of oat varies from 5 % to 9 % with variations due to environment and cultivar.

Unlike other cereals, most of the lipids in oat are found in the endosperm. Hence, oat flour which is predominantly the starchy endosperm material has a high lipid content compared to other flours [21]. The high lipid content of oat flour can lead to rancidity

during storage due to the unsaturated fatty acids and a highly active lipase component [14, 46, 47]. Table 2.20 shows that the starchy endosperm (which comprises the largest proportion of the oat grain) typically contains over half of the lipids present. The concentration of lipid in the starchy endosperm of oat is reported as typically 7.0 %. [14]

Table 2.20: Lipid Distribution in Oat Grain [21]

Grain fraction	**Distribution** (%)
Embryonic axis	2.1
Scutellum	6.4
Bran	38.2
Starchy endosperm	53.3

Literature reports that free and bound lipids accounts for 5.5 % to 8.0 % and 1.4 % to 1.6 % of oat respectively [21]. Triglycerides account for 41 % to 80 % of the free lipids, depending on the sampling methods and extraction technique [21]. Some of the lipid components of oats a reported to have a high antioxidant activity [46, 47].

2.6.Introduction - Cereal Biopolymer Separation Processes

The following sections of the literature review summarise information recently published in literature on the separation of starch and protein from cereals, focusing on oats and relevant wheat processes. Other cereals are not discussed, unless specifically relevant to this research project. The focus in this part of the literature review is the processing steps that are involved in the separation of the starch and protein fractions in wet conditions. General upstream processes, such as cleaning, hulling, drying, cutting, rolling, and milling are briefly summarised for completeness (in the context of how they affect these downstream processes). Much of the following section, describing the various separation processes, is summarised from the material available in the commonly referenced, published texts mentioned at the start of this chapter.

There is a wealth of information published on the processing of cereals into various products, especially the processing of wheat. Commonly referenced texts have been listed previously [2, 6-9, 48]. There is some information published on the processing of oats, in particular the standard upstream processing used to prepare oat grain for manufacture into rolled oats. Much of the general information on oat processing is published in texts listed previously [1, 3, 5, 10, 11, 49]. Very little has been published on the Al-Hakkak Process and what has been published is related to this research project and undertaken before this study commenced [50-55].

2.7. Background

Proteins and starches are common industrial raw materials for specialty chemical production, which can be obtained from a range of natural and renewable sources such as cereals, legumes and root crops [48]. As discussed previously, the characteristics of bio-derived protein and starch vary, depending on the source. Some characteristics considered more desirable than others and as a result the value of a specific biopolymer fractions varies. As discussed previously, cereals contain mainly protein and starch. Minor components include soluble sugars, non-starch polysaccharides and lipids. However, these minor components can influence the protein and starch separation due to their natural functionality, such as emulsification, bio-conjugation (including Maillard cross-linking), rheology modification, and surfactant activity.

The extraction of biopolymers from cereals is an increasingly important and growing industrial activity, especially the isolation of protein and starch fractions [25, 26, 31, 56-60]. This is due to the increasingly widespread application of these biopolymers in food and industrial products. Wheat is a common source of two commonly used biopolymers: protein and starch. There are several common processes for production of these biopolymer fractions from wheat grain including the Martin Process, the Dough Batter Process, and variations of these. Compared to bread making and baking there is limited information published on the factors influencing wheat protein and

starch separation with much of the knowledge protected as trade secrets by individual industry manufacturers [25, 31, 59-65].

2.8. Upstream Processing

Most industrial processes for extracting biopolymers from wheat and oat use a raw feed material that has either been milled into flour or a grain that has been dehulled, cut, and/or rolled [66]. These initial, upstream, processing steps prepare the grain for the downstream extraction processes by cracking open the outer layers and exposing the starch and protein rich endosperm [49]. The upstream processes vary slightly between cereals and from factory to factory, but the general overall process remains the same and Figure 2.5 summarises these.

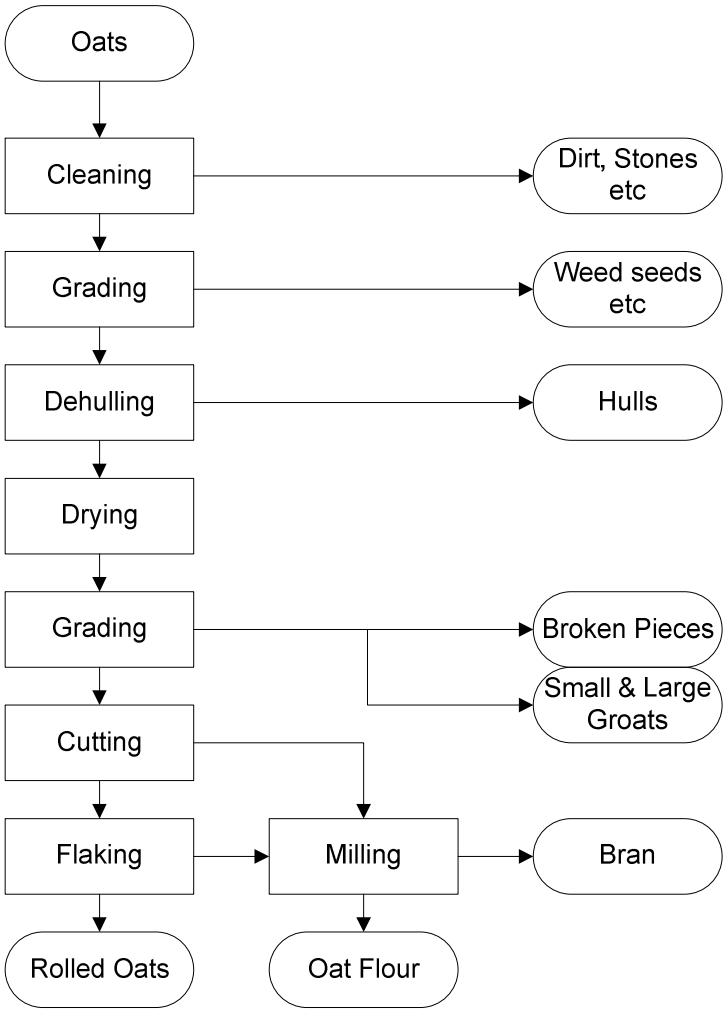


Figure 2.5. Typical oat processing (simplified) [10].

All cereals, including oat, are initially cleaned [6, 10]. Cleaning removes the unwanted materials such as metal, stones, weed seeds, dust, and dirt from the grain using a range of processing equipment such as separators, vibrating screens, aspirators, and de-stoners. A disc separator sorts the cereal kernels rejecting anything that has an incorrect shape.

Oat grains are then dehulled using specialist equipment to remove the outer shell of the kernel (hull) from the oat groat [10]. The lower density (lighter weight) hulls are then removed by air separation to produce a purified groat stream. The groats are further cleaned in a scouring process using equipment that brushes dirt and dust from the surface of the groats.

Most cereals are conditioned in a process called tempering where moisture is added to the groat to achieve an overall moisture content of around 15 % [6]. The groat is then stored for typically 8 to 24 hours and then blended to ensure an overall uniform composition. This tempering process toughens the bran and mellows the inner endosperm. This facilitates the separation of the bran and endosperm components during milling.

Oat is higher in lipids than wheat and other cereals [10]. A different conditioning process is used to reduce the moisture content and inactivate the lipase enzymes that are responsible for creating rancidity of oat during storage [10]. The oat groats are conditioned by adding water and heating in a kiln to a temperature of approximately 93 °C. This heating process helps to inactivate undesirable lipase enzymes present in oat grain, reducing their activity to typically 20 % to 40 % of the original activity. Following this, a drying process reduces the moisture level to an acceptable range for project storage (7 % to 10 %). The oat groats are cooled to less than 50 °C for storage.

For oat, the conditioned groats are graded with larger groats being separated from the small groats and broken pieces and then sent to a cutting process [10]. In the cutting process the groats are steel-cut and sifted to segregate the large, regular, and small pieces, which go into different products. For both steel-cut groats and uncut large groats, the groats are then steamed to increase the moisture and elasticity prior to the flaking process. The flaking process rolls the oat groats into flakes with the uncut

groats and various steel-cut groats fractions producing different end products. The flakes are then dried to typically 11 % moisture prior to packaging.

The tempered groat from cereals is milled or ground to produce flour [6]. For oat, both steel-cut and uncut oat groats are used to produce oat flour and the by-product oat bran [10]. Roll-stands and hammermills are typically used to grind the groats into flour with the end product specification (particle size) determining the extent of this milling step. The larger particles are separated from the smaller particles using sifting with nylon cloths or stainless steel screens. The bran is separated from the milled endosperm using sieving and air classification.

2.9.Starch and Protein Separation

The traditional processes starch and protein production from wheat flour typically rely on three key processing steps: 1) dough kneading and development, 2) extraction and separation of the protein and starch fractions (sometimes termed washing), and 3) isolation of the protein and starch fractions (usually using screening and/or settling and/or centrifuging technologies) [25, 56, 59, 63, 64, 67]. The starch and protein fraction are dried in separate processes. Step 1 (dough kneading and development) occurs once during processing whereas steps 2 and 3 occur several times to achieve the desired product purity specification. These steps are described in more detail for each of the different production processes later in this chapter. For wheat protein (gluten) and starch production it has been previously shown that varying the processing parameters for all three steps influences the final purity and yield of the wheat protein product [25, 63-65, 68].

Most industrial processes for wheat protein and starch separation take advantage of the unique functionality of the gluten proteins in wheat to agglomerate and form a cohesive protein network [25, 58, 59, 63-65, 68, 69]. It is this gluten protein network that gives wheat dough the visco-elastic rheological properties. The gluten protein network allows the insoluble starch granules and soluble biopolymer fractions to be readily removed from the insoluble gluten protein. The gluten protein network is

initially formed during step 1 (dough kneading and development) and can be affected by both the composition of the raw material (the flour) as well as processing factors such as kneading conditions and extraction conditions.

2.9.1. Gluten Protein Network

Gluten protein network is the term used for the three dimensional, visco-elastic network of protein molecules that forms when water is added to wheat flour, and the prolamin (gliadin) and glutenin proteins interact to form a cohesive network [2, 6, 70]. Only the insoluble gliadin and glutenin proteins from wheat, not the other cereals, have this network-forming activity [2, 6]. It is this interaction that makes wheat flour useful for bread making. When water is added to wheat flour, the gluten proteins interact to form a cohesive, three dimensional network [71, 72]. Applying mechanical work by kneading provides the necessary energy and extends the network into strands and films creating a cohesive, visco-elastic, gluten protein enriched mass [25, 71, 72]. This agglomeration of the gluten proteins in wheat is usually termed “dough development” [25, 34, 73].

Gluten agglomeration has been the focus of many studies, but, despite this, it is not fully understood at a molecular level. It generally agreed that during the initial dough mixing and subsequent dough development the following sequence of events occurs [6, 34, 70, 73]. Following water addition and the commencement of mechanical mixing the dry flour particles start to absorb the water. Under the microscope, the flour particles appear to explode and strands of protein are expelled into the water. As mixing progresses, new dry surfaces are exposed to water and the protein hydrates further. As the protein network is softened by hydration, the starch granules become less firmly imbedded in the protein and are able to move around separately to the protein. With further mixing dough development occurs and the dough changes consistency from a slurry into a smooth, cohesive, visco-elastic mass. It is during this stage that the gluten proteins (the glutelins and gliadins) begin to interact at a molecular level to form the gluten protein network and bonds between the proteins are formed, broken and reformed.

There has been much discussion in literature over many years on the formation of the gluten protein network. Recent publications agree that the gluten protein network is held together by both inter- and intra- molecular bonds (mainly by disulphide linkages) but also secondary bonding forces such as hydrogen bonding, ionic bonding and other non-covalent bonds [25, 71]. However, the exact mechanism is still under considerable debate [74-76]. The degree of agglomeration can be measured using simple, standardised, wet sieving methods or more complex centrifugation methods [25, 72]. It is suggested that a resting period (immediately following kneading) allows the bonds to rearrange to minimise internal stresses in the dough. New bonds then form resulting in the development of a gluten network with enhanced strength [25]. Over mixing is reported to break the newly formed bonds between the molecules and weaken the gluten network, although some authors report that gentle remixing of over mixed dough can restore the gluten protein network [64, 77].

Recent literature indicates that the glutenin fraction plays a key role in the formation of the gluten protein network with the insoluble, high molecular mass subunits displaying a strong correlation to gluten protein network formation [78]. Literature suggests that the native gliadin proteins are present as irregular globular structures interact to form agglomerates with the glutenin proteins when water is present [71]. This literature review has established that the mechanism for gluten protein network formation in wheat dough is not yet well understood or characterised [70, 71].

Recently published literature proposes a particle model for gluten hyper-aggregation [79-82]. This model is gaining general acceptance, as it accounts for the measured characteristics of the gluten at various scales. It proposes that glutenin chemically interact to form particles of 10 μm to 100 μm diameter which are the building blocks of the gluten protein network. The model proposes that hyper-agglomerates of these building blocks form (100 μm to 1000 μm diameter), based on physical interactions which vary with processing conditions.

An alternative model discussed in literature is the loop and train model [83]. This model proposes that the glutelins exist as long polymer strings with unbonded mobile regions (loops), interspersed with regions of bonding between subunits (trains).

From a manufacturing point of view, wheat gluten (gluten) is a key co-product of wheat starch production with typically one tonne of gluten produced per 6 tonnes to 7 tonnes of starch [25, 48]. Gluten production offsets the high cost of starch production [48]. However, wheat gluten is typically only 75 % pure, as individual starch granules are embedded in the gluten protein network [71]. Wheat gluten can be isolated with little or no loss of the inherent protein network-forming activity [25]. This co-product is typically sold in the form of flour as "vital wheat gluten" and is commonly added to wheat flour to improve the dough forming and baking characteristics.

2.9.2. Wheat Starch and Protein Separation

There are a number of well-known, industrial processes used to separate wheat protein, starch and the other biopolymers from wheat and these have been comprehensively reviewed by Borght et al. [25]. Most of these technologies are operated as "trade secret" processes and whilst information is available on the overall process, there is little detailed information available on the effectiveness of the different processes. Most of these processes use wheat flour as the raw feed material, although some use the grain that has been dehulled and/or rolled.

Dough Process (Martin Process)

Arguably the most well known process for the separation of wheat gluten and starch is the Dough Process (also called the Martin Process) which has been widely used on an industrial scale since 1835 [25, 84]. In the Martin Process, a stiff dough is formed (40 % to 60 % water added by weight of flour), which is kneaded then set aside for a period of time to allow the gluten protein network to relax (termed "dough resting"). Once rested the dough is then washed by kneading in water to remove an aqueous slurry containing the insoluble starch granules and the soluble water extractable fraction. This slurry can then be separated using either sieving, centrifuging, or tabling (a settling process) into A-starch (intact starch granules), squeegee starch (also called B-starch, a low quality starch, often comprising damaged starch granules), and the water extractable fraction. Mixing temperatures in the range 24.5 °C to 28.0 °C

generally give optimum gluten network formation with higher temperatures resulting in a more rapid development of the gluten network [25, 85]. By mass of flour, yields of 40 % to 60 % starch are typically achieved with approximately 80 % of the total starch recovered as A-starch. The remaining starch is either embedded in the gluten protein network and is not extractable or it is lost as squeegee starch.

In the Martin Process, the control of the protein-protein and protein-starch interactions during washing is important [25]. The protein-protein interactions must be maintained and if possible enhanced, while the protein-starch interactions need to be weakened and minimised. The separation of the starch and gluten has been shown to be dependent on the water content of the dough, with the optimum content varying with variations in wheat flour [60]. Separation improves as the water to flour ratio increases from 0.60 to 0.95, but decreases from 0.95 to 1.10 [77].

Dough-Batter Process

The Dough-Batter Process is essentially a variation of the Martin Process and is widely used on an industrial scale for the separation of starch and protein from wheat [25, 84, 86]. A stiff dough is formed, similar to the Martin Process. After resting the dough is partially dispersed in additional water by vigorous mixing and the resulting batter is sieved and/or centrifuged to separate the starch and protein fractions. Yondem-Makascioglu et al. [68] report that dough mixing trials over the temperature range 20 °C to 50 °C resulted in reduced squeegee starch mass at lower temperatures and slightly increased protein recovery as the temperature increased combined with faster washing and sieving processes. However, mixing at 50 °C resulted in a highly dispersed dough, with very poor protein recovery. Increased mixing time and speed (greater energy/work) have been found to improve gluten network formation, although over-mixing results in weakening the gluten network as the bonds between the molecules are ripped apart [64, 77, 87]. At high mixing speeds, dough development and, hence, gluten agglomeration has been shown to be independent of mixing [87]. By mass of flour, yields of 69 % to 79 % starch and 11 % to 14 % protein are typically achieved [25]. Increases water content are reported to correlate with improved protein agglomeration [64].

Batter Process

The Batter Process is another process commonly used at industrial scale for the separation of wheat starch and proteins [25]. A slack batter is formed (about 100 % water added by mass of flour depending on the flour quality) which is mixed and allowed to rest [25, 88]. More water is added and the dough is then broken apart by vigorous mixing to form “curds” of protein agglomerates suspended in starch milk [25, 88]. The protein is recovered by sieving and the starch is purified by either tabling (settling) or centrifuging. Three-phase decanters are currently used to separate the A-starch, B-starch, protein, and water soluble fractions [88]. Typical mixing temperatures are in the range 40 °C to 55 °C to enhance gluten network formation and minimise processing time [86]. Typically, by mass of flour, yields of 68 % to 77 % starch (with about 3 % protein contamination) and 7 % to 13 % protein are achieved [25]. Knight and Olsen [60] report gluten recovery increasing with decreasing water to flour ratios with the optimum flour to water ratio dependant on the flour characteristics.

Fesca Process

The Fesca Process is essentially a variation of the Batter Process, using different operating conditions [25]. Flour is rapidly mixed with water to form a batter, with the temperature controlled to 30 °C to minimise gluten agglomeration [25, 84]. The starch, squeegee starch, protein, and water extractable fraction are separated using centrifugation. The protein water extractable fraction are contained in the aqueous supernatant [25]. Typical mixing temperature of 30 °C is used to minimise gluten network formation [89]. Typically, by mass of flour, yields of 56 % to 78 % starch (with about 1 % protein contamination) with 63 % to 87 % of the total starch recovered [25]. The protein rich stream is typically only 20 % to 40 % protein (dry mass) [25]. Too much and too little water negatively impacts on separation, with the optimum flour to water ratio dependent on the flour characteristics [60].

Rasio Process

The Rasio Process is another variation of the Batter Process [25]. Flour is rapidly mixed with water to form a batter that is homogenised to give a uniform suspension, with no gluten agglomeration [25, 31, 88]. This suspension is centrifuged to separate the starch from the protein rich fraction and the protein rich fraction is then processed through a pin mixer, where agglomeration takes place and the protein agglomerates are then separated from the aqueous stream using screens. The starch is almost entirely squeegee starch and the protein rich stream is typically only 80 % protein (dry mass) [25, 31].

Protein Hydrolysis Processes

Hydrolysis processes involve chemically modifying the protein by partial hydrolysis (solubilising) using sodium hydroxide, ammonia or enzymes [25, 90-93]. The starch is typically separated from the extract liquor containing the hydrolysed proteins by either centrifugation or tabling. The functionality of the chemically modified protein is significantly changed; in particular, the solubility is increased. Typically 70 % to 80 % of the total starch is recovered.

Whole Grain Separation Processes

Other wet based separation processes have been developed that use the whole wheat grain, rather than wheat flour, including Halle Fermentation, the Alsatian Process, the Longford-Slotter Process, Pillsbury Hydro-milling and the Far-Mar-Co Process [25, 84]. These technologies have not been widely adopted by industry [25].

Shear Based Separation Process

A novel method for mechanically separating wheat starch and gluten has been reported in literature which exploits the different rheological properties of the gluten protein (visco-elastic) and the starch (dilatant at low water content) [56, 58, 67, 72]. The authors report that separation was achieved by applying a curved shear field to wheat dough to generate a starch rich and protein rich fraction. The authors highlight that the

key advantage of this method was the low water requirement. To date investigations using method have only been reported at laboratory scale.

2.9.3. Processing Factors

A number of factors are known to affect the separation of wheat starch and protein in industrial processes. Borght et al. [25] reviewed recent literature publications and provided a comprehensive summary of these factors. Below is a summary of the main factors that affect starch and gluten separation using wheat flour.

Flour

Flour with good bread making properties have also been found to result in high gluten yields in the Dough Process [94]. Some studies have found that flour that performs well in bread baking also displays good gluten extraction in the Dough-Batter Process, but other studies have not found this [25]. Composition differences (such as starch, protein, sugar, and lipid) have been reported to influence extraction, with flour that performs poorly in bread baking reported as having higher levels of undesirable squeegee starch [65]. The particle size distribution of the flour reportedly affects the protein agglomeration, with high amounts of small particles slowing the agglomeration process [31, 95, 96]. Mechanical processes used in producing flour (milling) have been shown to result in damage to the starch granules resulting in more squeegee starch [26, 31].

Protein

In general the higher the protein content, the higher the gluten yield [94, 95]. Variations between cultivar, and hence composition have been found to affect gluten extraction and purity [59]. High levels of glutenin aggregation correlate with high protein yield and reduced starch contamination in the dough batter process [59, 97]. Age has been shown to have a negative impact on the properties of the gluten in wheat flour [31].

Starch

Starch granules are physically entrapped in the protein network, resulting in starch contamination of the protein and reduced starch yield [98]. Aging has been reported to negatively impact the separation of the starch, with the undesirable squeegee starch fraction increasing with age [99]. The severity of the grinding (milling) process has been shown to increase starch damage, resulting in a higher levels of squeegee starch [100].

Non-Starch Polysaccharides

Non-starch polysaccharides particularly arabinoxylans, are reported as having a negative effect on starch/gluten separation [59, 69, 97]. This has been attributed to the viscosity modification effects of arabinoxylans and it has been suggested that some arabinoxylans act as a physical barrier to gluten protein interactions, inhibiting the formation of the gluten network [69]. Incorporation of arabinoxylans in the gluten protein network appears to be due to entrapment, rather than molecular interaction [97].

Lipids

Lipids change as flour ages to form free fatty acids which can affect separation of the starch and protein [65]. Lipids are known to interact with the protein present in flour during protein agglomeration processes [9, 59]. The removal of lipids from wheat dough has been shown to increase the strength of wheat dough [101].

Process Aids

There are a number of process aids used in starch gluten separation such as ascorbic acid, sodium chloride, enzymes, and viscosity modifiers. A recent study reported that ascorbic acid had no effect on the separation behaviour of starch and protein [65]. Sodium chloride (common salt) has been shown to improve separation [102]. Enzymes that catalyse reactions, particularly hydrolases specific to the gluten protein that allow the dough to relax have been shown to affect the starch protein separation.

These hydrolases are reported to significantly increase the processing and separation properties, resulting in improvements in protein agglomeration. [87, 95, 96, 102-104]. The viscosity modification properties of xylanases reportedly affect the gluten protein agglomeration [69]. The addition of cellulose and proteases have been shown to significantly increase the protein agglomeration [103]. Reducing agents can affect viscosity and dough development by reversibly breaking down the gluten bonds during mixing and then later allowing the gluten bonds to re-form [87]. The addition of the amino acid cysteine has been shown to weaken wheat dough [105, 106]. Salvador et al. [107] found the addition of salt, sugar and yeast altered the rheology of the dough, suggesting that the additives influence the gluten protein network. Schurer et al. [106] found that the addition of urea and salts weakened the gluten in ambient conditions.

2.9.4. Starch and Protein Purification

In general, industrial starch separation processes use either size based (screen and sieves), or density based (centrifugation and tabling), methods to purify the starch and protein streams.

Size based techniques use sieving to separate larger agglomerated protein particles from the smaller starch particles. Other processes are density-based and apply centrifugal forces to separate the denser starch fraction from the protein fraction. A few processes employ a combination of size and density based separation [25, 71]. A novel method for the separation of starch and protein that involves applying a shear force to dough is being developed at the University of Wageningen, Holland [56, 58, 72].

For density based centrifugation methods, the bottom stream contains the A-starch and the top stream contains the gluten and the B-starch (squeegee starch) [25]. The squeegee starch contains the water unextractable non-starch polysaccharides, some proteins, ash as well as the damaged starch granules and is typically gelatinous at room temperature. Starch tabling is another density based separation technique where the starch and gluten flow over an inclined table, allowing the starch to settle and the protein and water extractable material to be carried away in the water stream. The squeegee starch settles on the surface of the A-starch and is scaped off.

The gluten rich protein is typically separated from squeegee starch after centrifuging using screens to separate the large gluten agglomerates from the smaller squeegee starch particles [25]. Pre-screening to remove the gluten agglomerates followed by centrifuging of the starch milk produces a purified A-starch stream.

2.10. Oat Biopolymers

2.10.1. Uses of Oat Fractions

Oat hulls, a by-product of rolled oats and oat flour processing, have been used for the production of fufurals and furan related compounds [1]. Oat hulls have also been used in the production of phenolic based resins and adhesives, carboxymethyl zylans, other pentosan compounds, as well as culture medium for yeasts and fungal based proteins.

Oat flour has been found to have high antioxidant capacity and this attribute has been used in various applications [1, 12]. The antioxidants present in oat have been found to include tocopherol, hydroxycinnamic esters or long chain alcohols, ω-hydroxy fatty acids of glycerol, 5-avensterol and avenathramides [12].

Aqueous slurries of oatmeal and oat flour have also been used in cosmetic products, such as facial masks and soaps [12] and have been found to have soothing effects when used in the treatment of various skin conditions [1]. Various extracts of oat have been investigated for use in pharmaceutical applications such as follicle stimulation, cholesterol lowering, and ulcer prevention properties [1].

Oat contains many enzymes. However, these are at such low concentrations that extraction is not considered economic [1]. Oat gums have a wide range of applications such as stabilisers, thickeners and emulsifiers [1].

Oat proteins have a number of functional applications. Oat proteins have been found to have good binding and foaming properties (albumins), and chemical modification such as acylation improves the solubility and emulsifying properties [1].

2.10.2.Existing Oat Separation Processes

Whilst oat is high in protein, the type of protein present in oat lacks the network-forming activity of the wheat gluten proteins [66, 108]. This means that the separation processes that rely on the gluten protein network in wheat cannot be applied to oat. Some studies have been undertaken into the separation of the starch, protein and lipid components of oat [1]. This review found that the methods for oat fractionation typically rely on chemical modifications (such as enzyme digestion) to carry out the separation and purification of the biopolymer fractions.

The exception is a new and novel process called the Al-Hakkak Process that is similar to the Martin Process used for the separation of wheat starch and proteins [52]. This patented process forms the basis of the research undertaken for this research study and will be discussed in more detail later.

Bran Extraction

Extraction processes exist for the separation of biopolymer fractions from oat bran, such as the Ostar Areevin ^{TM} process [109, 110]. Oat bran is high in oil, and oat bran that was separated in the rolling and/or milling process was the feedstock for the extraction of lipids/oils from the other components. Such extracts were reported as typically dark in colour and not homogeneous [109]. A patented process to extract biopolymers from oat bran used aqueous alcohol extraction [110]. The crude extracts were recovered using expensive ion-exchange chromatography, resulting in a very small yield. Multiple product streams were generated from another patented process for the extraction of biopolymers from oat bran [93]. This process involved heating a slurry containing the bran rich fraction in aqueous, alkaline conditions to extract the soluble fraction containing protein, gum and colloidal starch. This soluble fraction was cooled, to precipitate the protein which was then removed, along with the starch to generate a gum rich supernatant.

Solvent Extraction

There are established solvent extraction process used to extract biopolymers from oat and other cereals. One patented method used either chloroform or ethanol as the solvent [111]. Concentration was carried out using evaporation followed by further downstream processing using alcohol. Another alcohol based process has been developed whereby a two-stage extraction process is used [112]. In this process biopolymers were extracted from oats using ethanol or propanol. Purification of the crude extract was achieved by further solvent extraction using methanol followed by evaporation. A process for the extraction of the biopolymer, avenanthramide, has been developed [113]. This process involved solvent extraction using aqueous alcohol mixture (ethanol, methanol, propanol and/or butanol) followed by pH adjustment and two-stage membrane filtration to purify and concentrate the crude extract. A process was reported for the separation of oat flour from oat gum using solvent extraction, but further separation of the flour into other biopolymer fractions (protein and starch) was not considered [114].

Solvent extracts using alcohols, ethers, esters, and mixtures of these, are reportedly unstable and will often separate into aqueous and oil phases, as well as soluble and insoluble fractions [113]. In addition, solvent based technologies are not considered "green" by the general public. With an increasing focus on "green" production processes in recent years, using these solvent extracts is becoming increasingly undesirable.

Carbohydrate Enzyme Digestion

Investigations are reported in literature by various authors into enzymatic hydrolysis of oats to solubilise the carbohydrate fraction, leaving behind an insoluble protein fraction. This literature review has identified processes that produced enzymatically hydrolysed starch from oats [115, 116]. Crushed oats were enzyme treated to solubilise the starch to form maltodextrin which was separated from the insoluble protein rich fraction. A patented process for the extraction of a carbohydrate/sugar based biopolymer fraction from oats involved enzyme digestion [117]. Following the initial enzyme digestion, cooking, filtration, and concentration (evaporation) process

steps were used to produce a refined extract. A patented process that used the inherent enzymatic activity of the oat seed to decompose the cell wall material and separate the endosperm was developed [118]. Further separation of the biopolymer fractions was not considered.

Protein Enzyme Digestion

This literature review has identified various processes that use enzymatic hydrolysis of the protein fraction to generate a soluble protein fraction. A process treating aqueous oat slurries with an alkaline pH with a protease was reported to generate an aqueous fraction that is stable, soluble, and protein rich [92]. The protein rich fraction was then neutralised and concentrated. Partial protein hydrolysis was used in some reported methods, where 40 % to 60 % of the total protein present in the oats was hydrolysed using the protease trypsin [119, 120]. A process for the isolation of oat starch from oat flour using protease digestion was also reported [121]. In this process an aqueous slurry containing oat flour was treated with the protease for 3 hours and the treated slurry was then centrifuged and the supernatant and tailings discarded. The starch fraction was washed with water then filtered and centrifuged prior to drying at 40 °C. A method for the extraction of soluble oat proteins with low lipid contamination was identified [122]. This method used an aqueous, oat flour slurry that was treated with a protease enzyme (selected to have minimal amylase activity) in alkaline conditions that generated a soluble protein rich fraction and insoluble fraction. Centrifuging was used to separate insoluble material from the soluble supernatant, which also contained colloidal material that was not easily removed. The supernatant was concentrated (by evaporation or membrane filtration) to a concentration of 5 % to 15 % total solids and then separated using physical separation (such as centrifuging or membrane filtration) to remove the fine colloidal solids. The final polished supernatant/permeate contained up to 30 % total solids.

Lipid Enzyme Digestion

A patented process for the removal of lipids from oats used enzyme digestion to solubilise the lipid fraction of the endosperm using microbial lipases [123]. This

process also used surface active agents (surfactants) to disintegrate the aggregated starch granules and reduce the particle size distribution of the starch.

Protein and Starch Enzyme Digestion

Methods are reported that produce enzymatically hydrolysed protein and starch from oats using two-stage processes [90, 91]. In these processes crushed oats were enzyme treated using endopeptidase, to solubilise the proteins which were recovered as a soluble fraction. The resulting starch rich solids were then solubilised using a carbohydrate specific enzyme. Another process used enzyme digestion as a final purification of the protein rich stream [124]. In this process an aqueous oat slurry was separated into a heavy fraction containing starch granules and a light fraction containing starch granules entrapped within the protein network. The light fraction was then heated (above 120 °C) to gelatinase the starch and breakdown the protein network. The resulting solution was cooled and treated with alpha-amylase and beta-glucanase enzymes to separate the protein precipitate from the clear soluble supernatant. The supernatant was then further treated with amylo-glucosidase or fungal amylase enzymes to saccharify the carbohydrates present.

Alkaline Hydrolysis

Alkaline hydrolysis of oats to solubilise the protein fraction was reported in literature by various authors [125-128]. In one process protein was solubilised in an aqueous based process at pH 9.2, using sodium hydroxide and a solid:solvent ratio of 1:6 [125]. The alkaline slurry was sieved to remove the course bran and centrifuged to remove the fine starch granules which are then dried. Hydrochloric acid was added to the alkaline supernatant to lower the pH to 5.0 and precipitate the protein isolate. This acidic slurry was centrifuged to separate the protein solids from acidic supernatant and then both the protein solids and aqueous, acidic supernatant are dried. A second alkaline wash was undertaken prior to acidification to improve the overall yield.

A process was reported that used either sodium hydroxide or calcium hydroxide to solubilise the protein by raising the pH of an aqueous oat slurry to pH 10.5 or pH 11.0 respectively [121]. The alkaline solution was stirred in low shear conditions for

30 minutes at 25 °C then centrifuged to separate the starch solids from the supernatant containing the solubilised proteins. The starch solids were then mixed with water and filtered prior to being neutralised using hydrochloric acid and centrifuged a second time. The starch solids from the centrifuge were further washed with water and centrifuged a third time prior to drying at 40 °C.

Mechanical Separation

A process for the isolation of oat starch from oat flour using high shear in water has been developed [121]. Oat flour was soaked in water at 20 °C for 6 hours, prior to processing in a tissue homogeniser for one minute. The resulting slurry was centrifuged and the supernatant and tailings (containing the protein and soluble sugars) discarded. The starch sediment was washed in water then filtered and centrifuged a second time, prior to drying at 40 °C.

2.11. Al-Hakkak Process

2.11.1. Overview

The Al-Hakkak Process is a patented process for the isolation of starch and protein from plant materials, including oat flour, using gluten. This process is the topic of this research project [51, 52].

The process uses similar processing steps to the Martin Process for wheat protein and starch separation (Figure 2.6). The process starts with the addition of gluten protein (in the form of “vital wheat gluten” flour) to non-wheat flour such as oat flour. Water is added to form a stiff hybrid dough which is then kneaded. The added gluten proteins appear to promote agglomeration of the proteins to form a relatively stable gluten protein network. Similar to the Martin Process the hybrid dough is allowed to rest (stand undisturbed) to allow the protein network to completely form. The hybrid dough is then “washed” to extract the starch granules and transfer the starch to the wash water. This is done by adding water and kneading the dough by hand in the

water. This is repeated several times until the wash water (starch milk) is relatively clear. This washing separates the insoluble protein network from the wash water which contains the starch granules. The wash water is filtered and centrifuged to purify the starch prior to drying.

The laboratory developed Al-Hakkak Process is the focus on this research project, which investigates methods of separating the starch and protein fractions from oat using industrially scalable processes to produce a uniform product. At the start of this current study the Al-Hakkak Process had only been developed and carried out on a small scale in the laboratory. Many of the processing steps had been established using laboratory scale techniques which did not readily scale up to commercially feasible, industrial scale processing. Methods for undertaking this extraction on an industrial and commercially feasible scale had not been studied. Significant research was required to identify and develop industrially scalable processes, robust enough to overcome the variations in raw material composition, whilst producing a uniform biopolymer product. These scale up considerations have been the foci of this study.

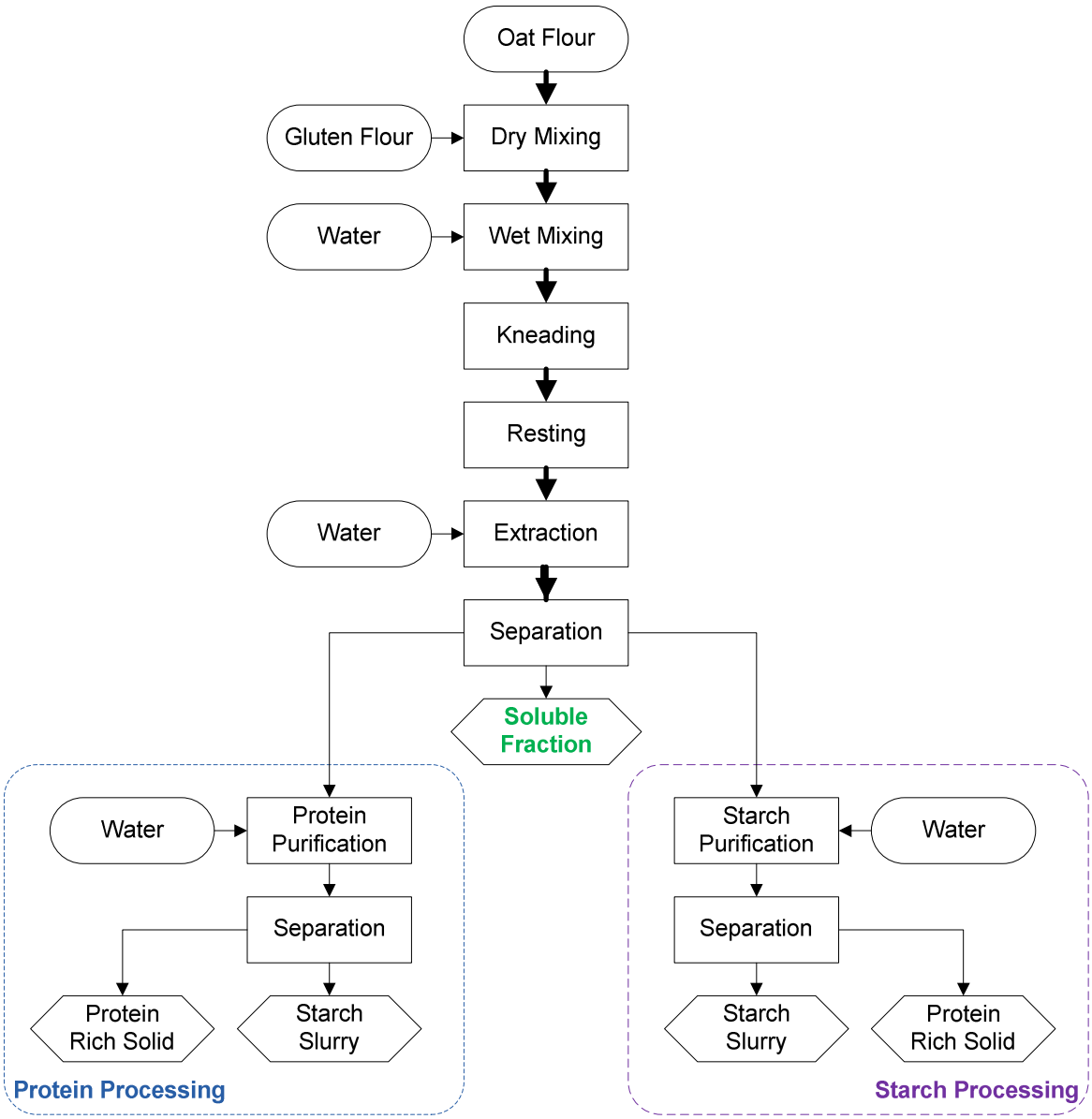


Figure 2.6. The processing steps involved in the Al-Hakkak Process.

2.11.2.Detailed Description of the Al-Hakkak Process

A more detailed description of the Al-Hakkak Process at laboratory scale follows. It is specific for oat flour (the selected raw material for this research project) and is based on published and unpublished data from the laboratory trials previously undertaken [51, 52].

Dry mixing

In the laboratory trials, initially 47.8 g oat flour and 11.9 g wheat gluten flour were accurately weighed. Both were placed in the small scale kneading device used for the wet mixing and kneading stages of the process. A Farinograph mixer was used which is a two arm z-style mixer produced by C. W. Brabender Instruments Inc (Germany). This equipment is described in more detail in Section 3.2 of this thesis. The samples were then mixed for 30 seconds using the Farinograph mixer.

Wet mixing and kneading

With the agitator running, 38.6 g of distilled water and 1.8 g of 2 % sodium chloride solution (both at 20.0 °C) were added over a 30 second period. Initially the contents of the vessel were not uniform, comprising lumps of dry flour surrounded by sticky dough. The sides of the Farinograph were scraped down by hand using a plastic spatula to remove any sticky material adhered to the side walls as mixing proceeded. After approximately 30 seconds all of the water was incorporated and stiff dough was formed that was not sticky to touch. During wet mixing the water was absorbed into the flour. It was considered likely that the oat and gluten proteins become hydrated and some of these proteins begin to interact and agglomerate. A lot of research has been undertaken on wheat protein hydration and agglomeration (as discussed previously). However, at the start of this research project no investigations had been undertaken to investigate protein hydration and agglomeration in hybrid oat-gluten dough.

At this point wet mixing was considered complete and kneading commenced. The transition from wet mixing to kneading was gradual, with no precise demarcation point separating the processes. Kneading continued for approximately a further 60 seconds (total wet mixing time). During kneading the oat-gluten dough became smoother in appearance like wheat dough and it is thought that dough development was occurring. In wheat dough this mixing provides the necessary energy for the gluten proteins to interact and agglomerate via physical, covalent and non-covalent bonding to form a cohesive, insoluble, protein network (as discussed previously for wheat dough) [25]. It was likely that the hydrated gluten proteins in the oat-gluten dough interacted to form a

protein network. However, at the start of this research project no investigations had been undertaken into this protein network. It was not known what role, if any, the oat proteins had in the formation of the protein network.

Resting

The oat-gluten dough was removed from the Farinograph and placed in a plastic bag to rest at 20 °C for between 30 and 45 minutes. For wheat dough, this resting period allows further development of the dough to take place [25]. Stresses in the protein network reduce, typically referred to as "dough relaxation" (as discussed in Section 2.9.2). It was considered likely that the oat-gluten dough relaxed similarly. However, at the start of this research project no investigations had been undertaken to confirm this.

Extraction

The oat-gluten dough was transferred to a container (approximately 5 litre) containing approximately 1 litre of tap water. The volume of water was not accurately measured, thus the dough to water ratio was not known. The temperature of the water was not measured but was estimated to be about 15 °C. The oat-gluten dough was then kneaded by hand in the water and the starch granules separated from the dough and were transferred to the aqueous extract liquor. Soluble biopolymers present in the oat-gluten dough (such as soluble proteins, sugars, and salts) were also transferred to the extract liquor. After a few minutes the liquor turned a milky white colour, due to the white oat starch granules.

The extracted dough remained as a cohesive ball during the washing process. It is thought that this was due to the protein network formed during kneading and resting. This had not been confirmed at the start of this research project.

The extracted, protein-rich, oat-gluten dough was carefully removed by hand and the extract liquor was replaced with fresh water. The extracted oat-gluten dough was returned to the fresh water and the extraction process repeated up to five times.

Extraction was stopped when the liquor did not change colour, indicating that very little starch was being removed from the protein-rich dough in the final extraction.

Separation

At the end of washing the insoluble protein rich solid (referred to as “spent dough”) was carefully removed set aside.

The extract liquors were combined and passed through a nylon screen (approximately 45 μm hole diameter) to remove any small protein particle contamination in the starch rich extract liquor. These protein fines were discarded. The combined extract liquor was left to settle. The settling time was not defined and ranged from 1 to 20 hours. Most of the clear supernatant containing the soluble biopolymers was carefully decanted off to leave behind a concentrated starch and supernatant slurry. The volume of supernatant that remained and the concentration of the starch slurry were not measured. In the laboratory the soluble biopolymer containing supernatant was discarded.

Starch Purification

The starch slurry was centrifuged in a laboratory centrifuge at 3000 g for approximately 20 minutes. The resulting pellet had 2 layers, a lower white layer containing the starch and a thin brown layer thought to contain fine protein and bran particles. The mass of this brown layer was not measured but was estimated visually to be less than 1 % of the volume of starch. The starch pellet was compact and hard and the brown layer was readily scraped off the white starch layer with minimal disturbance of the starch. The remaining purified starch was then carefully removed and spread over a glass tray and oven dried overnight at less than 40 °C (lower than the onset of gelatinisation temperature reported for oat starch [23]). In the laboratory no further processing of the starch was carried out.

Protein Purification

In the laboratory no further purification of the protein was carried out. The protein was spread in a thin layer over a tray, placed in a plastic bag and frozen at -24 °C for several days. The frozen protein was transferred to a freeze drier where it was dried over several days. The freeze drying conditions were not recorded. In the laboratory no further processing of the protein was carried out.

3. General Methods

3.1.Introduction

Several general research methods were common to several sections of the research project. This chapter describes the research methods that were used throughout this study. These methods include both processing techniques and analytical methodologies. Specialist analytical methods used for a specific set of investigations included: scanning electron microscopy, confocal scanning laser microscopy, large deformation rheology, running SDS PAGE gels, and mass spectrometry. These methodologies are specifically described in the relevant chapters of this thesis and are not discussed here.

3.2.Equipment

Two different processing scales were investigated during this study. Small pilot scale processing trials were used for the preparation of small samples with variable composition and kneading conditions. Larger pilot scale processing trials were used to provide a single uniform dough sample for repetitive analysis. Both the small and larger pilot scale processing equipment was similar in operation to large scale commercial processing equipment. The general descriptions of each item of equipment used for the different processes involved in the Al-Hakkak Process are included in this chapter as part of the individual process description.

Specific operating conditions for each item of equipment varied depending on the investigations being undertaken. The specific operating conditions for each item of equipment are discussed in detail in the relevant chapter of this thesis.

3.3.Flour

The samples in this project were prepared using: oat flour, wheat gluten flour, and/or wheat starch. The oat flour was supplied by Harraways Ltd (2007) and stored in a sealed plastic bag in a freezer until used. Prior to processing, the oat flour was removed from the freezer and allowed to defrost at room temperature. The flour was then sieved through a 500 μm screen to remove any bran and large particles. The vital wheat gluten flour was supplied by Healtheries Ltd and stored in a sealed plastic bag in a freezer until used. Commercially available Edmonds brand wheat starch (manufactured by Goodman Fielder New Zealand Ltd) was purchased and stored in a sealed plastic bag in a freezer until used. The gluten flour and wheat starch were defrosted at room temperature, but were not sieved as both had already been sieved during manufacture.

3.4.Mixing and Kneading

The small pilot scale dry mixing, wet mixing, and kneading was carried out using a Farinograph mixer fitted with a 50 g kneading vessel (Figure 3.1) (located at the Plant & Food Research campus in Lincoln, New Zealand). This is a two arm, z-style, mixer produced by C. W. Brabender Instruments Inc (Germany), and is widely used to produce small samples of dough. Many modern, full commercial scale dough kneaders employ two arm kneading technology with a similar kneading action.

Figure 3.1. Internal view of the Farinograph mixer.

The larger pilot scale dry mixing, wet mixing, and kneading was carried out using a small, two speed, dough mixer fitted with approximately 5 kg capacity vessel and a single dough hook shaped "E" dough arm (Figure 3.2). This mixer was produced by Hobart Food Equipment (Australia), and is of a type widely used by industry. Similar mixers are produced by various manufacturers around the world and can reach capacities of 180 kg. The Hobart used in these trials was located at the Plant & Food Research campus in Lincoln, New Zealand.

Figure 3.2. The Hobart mixer.

3.5. Sheeting

Small scale and pilot scale sheeting was carried out in two stages. The first stage used a manual roller to reduce the oat-gluten dough to a manageable, uniform thickness using a stainless steel rolling pin and depth guides to control the height of the rolled oat-gluten dough. Two depth guides were used, 22 mm followed by 10 mm. The second stage used a commercial, manually operated, dough sheeter consisting of two stainless steel rollers, rotated by a manually operated handle (Figure 3.3). The gap between the rollers could be varied from the rollers touching (0 mm) to a maximum of approximately 5 mm.

Figure 3.3. Pilot scale dough sheeter.

3.6. Extraction and Purification

Extraction was carried out using 500 ml stirred, baffled vessels, with a pitched blade impellor. The impellors were designed and built at AgResearch Limited. The design is described below in Tables 3.1 and 3.2. Images of the vessel and impellor set up are shown in Figures 3.4 and 3.5.

Figure 3.4. Vessel and impeller arrangement

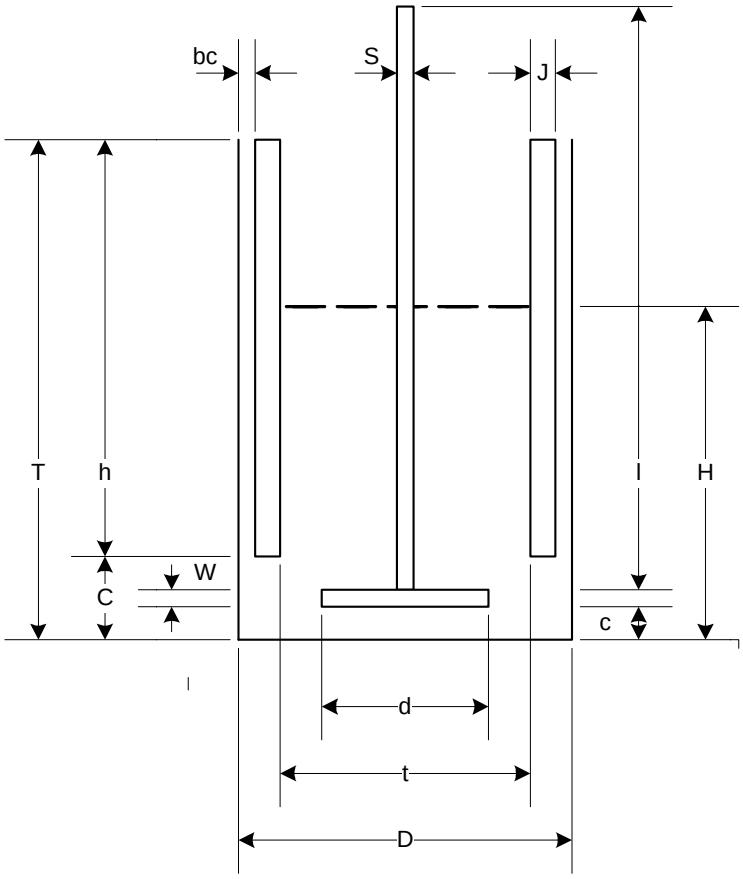


Figure 3.5. Impellor design.

Table 3.1. Impellor Design

Parameter	Symbol	Dimension	
Volume	V_A	600	ml
Working volume	V	250	ml
Tank total height	T	120	mm
Water level height	H	50	mm
Tank inner diameter	D	80	mm
Baffle-baffle tank space diameter	t	60.0	mm
Baffle length (3 baffles)	h	100	mm
Baffle wall clearance	b_c	0	mm
Baffle width	J	10	mm
Baffle bottom clearance	C	20	mm
Dough ball diameter	x	10	mm
Horizontal baffle-impeller clearance	v	5	mm
Impeller off-bottom clearance	c	5	mm
Impeller width	W	10	mm
Impeller diameter	d	50	mm
Shaft diameter	S	8	mm
Shaft length	l	~180	mm

Table 3.2: Vessel Design

Geometric ratios:	Actual	Literature [a, b]
D/T	0.67	-
D/H	1.6	1
b_c/T	0	0.014
J/D	10/80	0.083
C/J	2.00	0.25 - 0.5
v/x	0.5	1.5
c/D	5/80	0.25
W/d	0.20	0.2
d/D	0.63	0.40

a) R. R. Corpstein and J.B.Fasano. The High-efficiency Rotor to Liquid-Solid Agitation, Chemineer Inc, K.J.Myers, University of Dayton

b) Julian B Fasano and Andre Bakker. Advanced Impeller Geometry Boosts Liquid Agitation, Chemineer Inc, University of Arkansas

3.7.Gluten Protein Agglomeration Index

In traditional wheat starch and protein processing, such as the Martin Process and the Batter Process (briefly described in Section 2.9.2), the separation of the starch from the protein typically exploits differences in density and/or particle size of the starch granules and gluten protein agglomerates [25, 56, 59, 63, 64, 67]. As discussed in Section 2.9.4 of this thesis, the initial separation is commonly carried out using vibrating sieves. The generally larger agglomerated gluten protein particles are retained by the sieve, whilst the smaller starch granules pass through with the extract liquor. Centrifuging or tabling is then typically used to refine and purify the starch stream.

Methods for measuring the gluten protein agglomeration index are a widely applied technique for measuring the degree of gluten protein agglomeration [25, 59, 63, 64]. These methods are all similar and use a series of sieves with decreasing aperture size, usually 400 µm, and/or 250 µm, and 125 µm. Poor protein agglomeration produces a protein network consisting of smaller protein particles which can pass through some or all of these sieves. Good protein agglomeration produces large protein particles which are retained by the larger sieve. The gluten agglomeration index is measured by comparing the mass of protein retained by the larger sieve (400 µm) with the combined protein recovered from all of the sieves (400 µm, 250 µm, and 125 µm).

The method recently reported by Auger el al [63] was modified and used for these trials to assess the agglomeration of the oat-gluten protein solids from the Al-Hakkak Process. At the end of the extraction and purification process the extract liquor was poured over two stacked sieves (400 µm and then 125 µm). No additional water was used to flush the sieves. Any large protein particles that had formed were retained on the surface of the sieve. The sieves were drained for three minutes. During the draining period the sieves were gently agitated by hand to minimise blinding of screen surface by the wet protein solids. At the end of the draining period the wet solids sitting on the surface of each sieve (predominantly oat-gluten protein solids) were carefully removed using a plastic scraper and carefully placed into individual plastic containers. Each protein sample was weighed and then frozen over night. The frozen

protein samples were then freeze dried prior to analysis for total solids, ash and protein content.

3.8. Drying

Spray drying of the starch rich slurry was carried out using a Mini Spray Drier B-290 (located at the AgResearch Research Centre in Lincoln, New Zealand), manufactured by Buchi (Switzerland) as shown in Figure 3.6. This laboratory scale, bench-top system provided a method of trialling the feasibility of spray drying on a small sample of starch slurry. This equipment is suitable for proof of concept and assessing the spray drying conditions. Optimising the spray drying process would require trials to be run using a larger, pilot-scale spray drier [129].

The spray drier was operated following the method described in the operating manual. A brief summary of the operation of the spray drier follows. The starch rich slurry was placed in a glass conical flask and gently agitated using a laboratory magnetic stirrer to minimise settling of the starch granules. The starch slurry was fed into the spray drier at known flow rate using a peristaltic pump (part of the spray drier). The starch slurry was fed through the nozzle into the glass drying chamber. Preheated air for drying was also fed into the drier. The flow rate and temperature of the inlet air was controlled to an operator defined set point. The dried starch exited the drying chamber and passed through a small glass cyclone, to separate the starch particles, which were collected in glass vessel. The drier was operated by Rachael MacManus (AgResearch, Lincoln, New Zealand).

Figure 3.6. Buchi Mini Spray Drier B-290.

Tray drying of the insoluble oat-gluten protein product fraction and starch rich slurry was carried out in a laboratory oven with fan forced airflow. This oven provided good control of the air temperature (±0.2 °C) during the drying period.

Milling the tray dried samples was carried out using a laboratory scale Retsch Ultra-Centrifugal Mill ZM 200 in conjunction with a Retsch AS 200 Sieve Shaker (Verder Group, Netherlands), both located at the AgResearch Research Centre in Lincoln New Zealand. This mill uses both impact and shearing between the rotor and the fixed ring sieve for particle size reduction. The milling operation followed the method recommended by the mill manufacturer and a brief description follows. The feed material passed through the hopper onto the rotor where the initial impact pre-crushed the feed solids. These solids were then ground between the rotor and ring sieve. The particles passed through the ring sieve and were collected in the cassette surrounding the grinding chamber. The shaker was operated with a three sieve stack (32 μm, 63 μm and 75 μm) and all particles greater than 63 μm returning to the mill for further processing.

3.9.Starch Granule Size Measurement

The particle size distribution of the oat starch granules in an aqueous dispersion was measured using a Mastersizer 2000 Particle Size Analyser (Malvern Instruments Ltd, United Kingdom) located at the Plant & Food Research campus in Lincoln, New Zealand. This equipment uses a light scattering technique, Mie Scattering Theory, to measure the particle size distribution of aqueous dispersions. The particle size distribution is automatically calculated by the inbuilt Mastersizer software from particle diameters (derived from the raw data) using an internationally accepted method (British Standard BS2955:1993). Aqueous dispersions were prepared from the spray dried oat starch samples, by mixing approximately 1 g dried powder in approximately 50 ml distilled water, at room temperature, until fully dispersed. Each dispersion was loaded into the Mastersizer which automatically took aliquots of the dispersed solution for measurement. Two replicate samples were prepared and analysed for each sample. The Mastersizer was operated by Fadia Al-Hakkak (Plant & Food Research, Lincoln, New Zealand).

3.10. Composition Analysis

The composition of the oat-gluten dough, oat-gluten protein product, extract liquor (clear supernatant) and dried starch samples were analysed to determine any differences in the composition between samples. It was not possible to analyse the composition of the dilute starch slurry or concentrated starch slurry as it was found to be difficult to get a uniform sample for analysis.

- Moisture content as a mass percentage was determined in an oven at 105 °C, using test method ISO 1666 Starch -- Determination of moisture content, oven-drying method (±0.05 %). Samples were analysed by Tess Engren and Matthew Paulik (AgResearch, Lincoln, New Zealand).

- Ash content as a mass percentage was determined at 900 °C, using test method ISO 3593:1981Starch -- Determination of ash (±0.02 %). Samples were analysed by Tess Engren and Matthew Paulik (AgResearch, Lincoln, New Zealand).
- Protein was defined as the nitrogen content multiplied by 6.25 and was determined as a mass percentage by Dumas Combustion Method using a LECO CNS-2000 Elemental Analyzer (±0.05 %). Samples were analysed by Lidia Motoi (Plant & Food Research, Lincoln, New Zealand).
- Carbohydrate content as a mass percentage was estimated by difference.
- Starch was not specifically measured, but included in the carbohydrate measurement.

4. Oat Starch Drying

4.1.Introduction

This chapter discusses investigations carried out into drying the starch fraction produced by the Al-Hakkak Process. Some of this research has been presented in a peer reviewed conference paper presented at Chemeca 2009, Perth (2009) and a poster paper presented at the 8th World Congress in Chemical Engineering, Montreal (2009) [130, 131]. The majority of this research in this project has been focused on the formation of the protein network in the oat-gluten dough, as this was identified early on as a key factor for the separation of the starch and protein fractions. Thus, later chapters in this thesis are focused on the protein network. Only this chapter focuses on processing of the oat starch fraction. Starch granule separation processes are well established in industry. As discussed in Section 2.9.4 commonly used methods include centrifuging and settling. This chapter investigates the spray drying the oat starch slurry stream separated using the Al-Hakkak Process.

The main product from the Al-Hakkak Process is starch as it typically comprises over 60 % of the mass of oat flour. Starch granules such as rice, wheat, and corn starch are normally sold as a dry powder product and manufacturing processes for these are well established in industry [132]. The oat starch from the Al-Hakkak Process is extracted in an aqueous starch rich slurry and this must be dried to produce a commercially desirable powder product [51, 52]. Thus it was important to establish if the oat starch slurry could be dried to form a powder without damaging the inherent structure of the individual oat starch granules. It was also important to investigate if upstream extraction and purification steps would influence the oat starch drying process and the characteristics of the resulting dried starch.

The aim of this work was to visually assess the influence of drying on the structure of the oat starch granules. The focus of the trials, and hence the information reported within this chapter, was to observe if there were relative differences in the physical appearance of the dried oat starch as a result of variations in the extraction, separation, purification, and drying processes. The desired outcome was a visual assessment of

the structure and arrangement of the oat starch granules that were processed using different conditions. There were two hypotheses for this work: 1) drying conditions could be controlled to produce a fine powder of individual, undamaged, oat starch granules, and 2) processing conditions involved in the pilot scale Al-Hakkak Process have negligible influence on the drying process and the resulting oat starch powder product.

4.2. Background

4.2.1. Scanning Electron Microscopy

Over many years scanning electron microscopy has been widely used to analyse the structure of dough and starch samples and is widely reported in literature [28, 30, 31, 33, 133-137]. Thus, images from scanning electron microscopy can be, and are, readily compared between different studies. In contrast to standard light microscopy techniques, scanning electron microscopy produces images with a significantly greater depth of focus, a higher resolution, and a greater magnification [136]. Scanning electron microscopy is often preferentially used for starch granule imaging as it provides more detailed images with a clearer view of the starch granule morphology and allows more accurate evaluation of granule size, compared to standard light microscopy [135].

Scanning electron microscopy requires samples to be dried, distributed into a thin layer and then coated with a thin layer of conductive material, such as gold, prior to imaging [33]. This limits the application of scanning electron microscopy techniques. For example, starch granule slurries samples must be dried and this alters the appearance of the sample. The samples from this trial are dry starch powders and, as such, are well suited to scanning electron microscopy techniques. Unlike confocal scanning laser microscopy, using colorimetric techniques to analyse for the different components in the sample is not possible with scanning electron microscopy due to the coating step [135]. The different components in a sample must be distinguished by other factors, such as shape or size [72, 138]. This can make it difficult to identify various

components in the sample if the morphology of each component is not significantly different.

4.2.2. Particle Size Distribution

For comparison between the dried starch powder samples, the particle size of individual starch granules was also measured. The light scattering technique, Mie Scattering, was selected as this has been widely used for measuring the particle size distribution of cereal flour and starch granules in other studies [135, 139-142]. Light scattering techniques measure the angle of deflection of a parallel beam of light off a particle and calculating the particle size. Smaller particles reflect light at a larger angle compared to larger particles. Mie Scattering assumes the particles are spherical and the scanning electron microscope images can be used confirm the validity of this assumption. The method used in these trials required the dried oat starch granule powders to be dispersed in water for measurement. A disadvantage of this method is that any structures in the dried material that are unstable in water will collapse. Thus any structures held together by water soluble materials will breakdown. Also, as discussed in Chapter 2, individual starch granules particularly damaged starch granules may swell and even dissolve when dispersed in water. This would change the measurement result.

4.3. Methodology

4.3.1. Sample Description

The dried starch samples in these trials were prepared following the Al-Hakkak Process [51, 52] as shown in Figure 4.1. Five sampling points were selected to provide information on the influence of prior processing, such as extraction, acidification (to enhance separation), and purification, on the structure of the dried oat starch granules. A description of the samples can be found in Table 4.1.

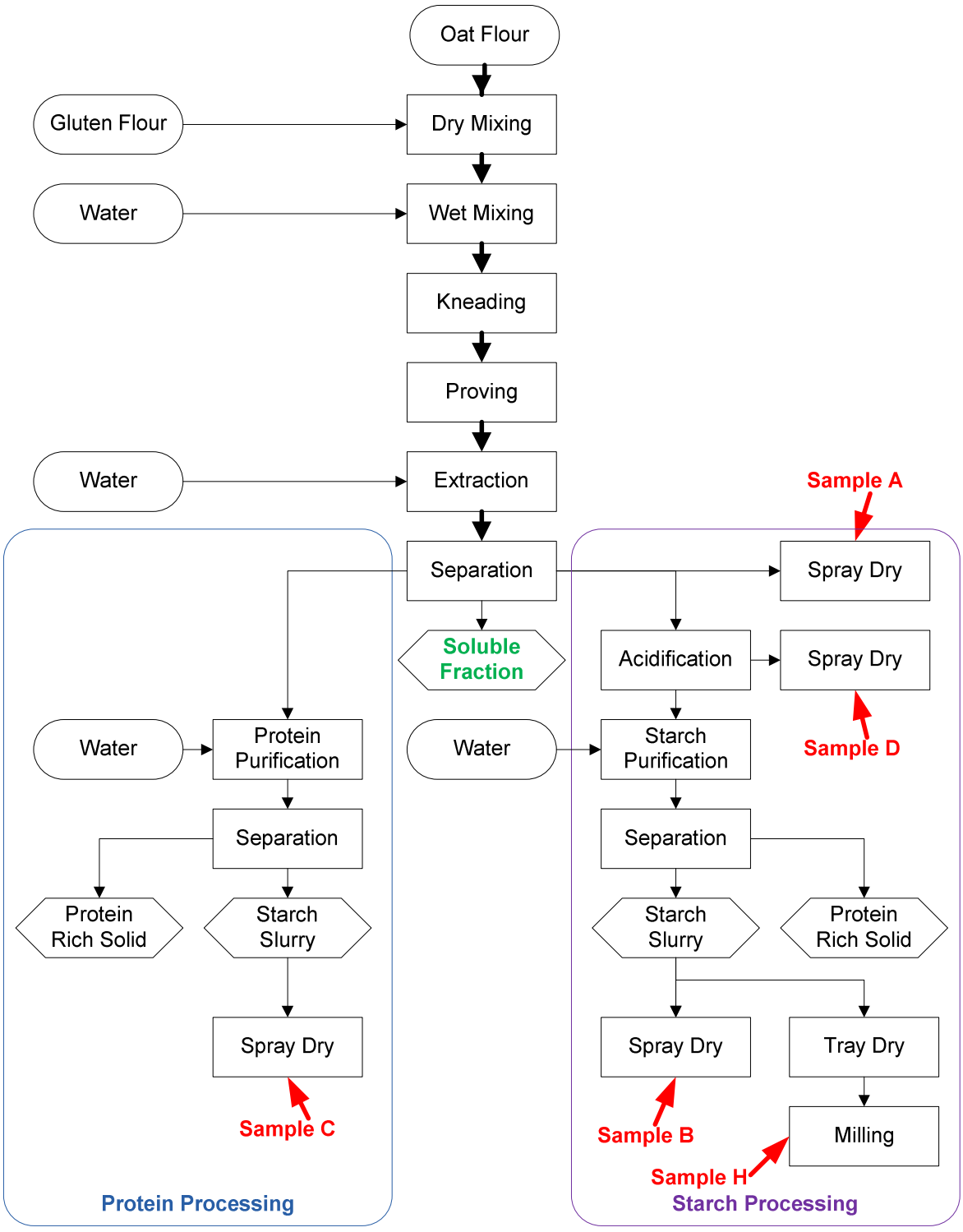


Figure 4.1. Schematic diagram of the Al-Hakkak Process showing the sampling points for the oat starch drying trials.

Table 4.1: Sample Description

Sample	Sample description
A	Spray dried starch slurry sampled after the first oat-gluten dough extraction stage.
B	Purified, spray dried starch slurry sampled after the starch purification stage, where the extracted starch slurry is then subjected to a starch purification stage.
C	Spray dried starch slurry sampled after the protein purification stage, where the protein enriched oat-gluten dough is subjected to a protein purification stage.
D	Spray dried starch slurry sampled after the first oat-gluten dough extraction stage that was acidified to facilitate separation.
H	Purified, tray dried and milled starch slurry sampled after the first oat-gluten dough extraction stage which was then purified using a starch purification stage.

4.3.2. Equipment

Samples were prepared using pilot scale processing equipment that is similar in operation to large scale commercial processing equipment. Dry mixing, wet mixing, kneading and extraction were carried out using a pilot scale, two speed, Hobart dough mixer fitted with approximately 5kg capacity vessel and a single dough hook ("E" dough arm) as described in Section 3.4. Spray drying was carried out using a Mini Spray Drier B-290 as described in Section 3.8. Tray drying and milling were carried out as described in Section 3.8.

4.3.3. Sample Preparation

All five samples were prepared from a single batch of oat flour and gluten flour. Preparation and storage of the oat and gluten flour used in these trials is described in Section 3.3.

Table 4.2 shows the dough recipe used to produce all of the samples in these drying trials. All of the samples were prepared from a single batch of dough using the same kneading and resting conditions as described in Table 4.3. The five samples received different extraction and purification processing to assess the influence of prior

processing on the structure of the dried oat starch granules. The extraction conditions used in this trial are described in Table 4.4.

Table 4.2: Dough Recipe

Parameter		**Mass**	**Dry Fraction** (%)	**Wet Fraction (%)**
Oat flour	g	480.6	80.1 %	47.7 %
Gluten flour	g	119.4	19.9 %	11.9 %
2 % NaCl solution	g	20.1	-	2.0 %
Water	g	386.4	-	38.4 %
Total dough mass	g	1006.5	-	-
Water temperature	°C	30	-	-

Table 4.3: Dough Kneading and Resting Conditions

Parameter		**Conditions**
Kneading temperature	°C	30
Kneading time	minutes	10 [a, b]
Resting temperature	°C	25
Resting time	minutes	90

a) Includes 30 seconds dry mixing prior to water and salt solution addition
b) The Hobart mixer used in this trial has two speed settings. The slow setting was used for the first four minutes to allow the flour and water to form a cohesive dough. Then the faster setting was used for the remaining six minutes mixing time

Table 4.4: Starch Extraction and Purification Conditions

Parameter		**First extraction**	**Starch purification**	**Protein purification**
Sample		A, D, H	B	C
Dough mass washed	g	980	Not measured	Not measured
Water mass	g	4000	4000	4000
Water temperature	°C	22	22	22
Wash liquor pH [a]		A = 3.78 D = 2.25 H = 3.52	B = 3.57	C = 3.98
Extraction time	minutes	90	90	90
Mixer speed	rpm	75	75	75

a) Measured at the end of the washing period

Samples A and D received the same single-stage dough extraction treatment. Both starch slurries were comprised of the extracted starch granules, the residual extract liquor, and contaminants (such as small protein particles). The extract liquor contained

the soluble biopolymers present in the original oat-gluten dough sample. Sample A was dried without further purification. Sample D was acidified as other published research suggested such conditions could enhance the separation of starch granules [143]. The separation and purification processes used for all of the samples are described later.

Samples B and H received the same two-stage extraction and starch purification treatment. The purification step removed residual insoluble protein particles contaminating the starch and removed the extract liquor containing the soluble biopolymers. Hence, there were less soluble biopolymers present in the slurry prior to drying. Sample B was spray dried. Sample H was tray dried and then milled to evaluate if this alternative and potentially less capital cost intensive process would be suitable for drying oat starch.

Sample C was the only sample taken of the starch slurry generated after the two-stage extraction and protein purification treatment. This starch was extracted from the insoluble protein network in a second protein purification step. These starch granules were more tightly trapped in the protein network and required this additional step to release them.

All of the starch slurry samples were stored in a fridge at 4 °C overnight before drying. For samples not receiving a further purification step (Samples A, C, and D) the dilute starch slurry samples were poured into trays and left to overnight (approximately 16 hours) in a refrigerator at 4 °C. During this time the starch granules settled to the bottom of the tray and formed a white layer beneath a clear supernatant. Approximately half of the volume of clear supernatant was carefully decanted off the top of the starch samples, measured and discarded. The starch granule slurries were then transferred to a sealed container ready for drying.

For samples undergoing a further purification step (Samples B and H), following extraction the dilute starch slurry samples were poured into trays and left to overnight (approximately 16 hours) in a refrigerator at 4 °C. During this time the starch granules settled to the bottom of the tray and formed a white layer beneath a clear supernatant. The clear supernatant was carefully decanted off the top of the starch samples,

measured and discarded. Distilled water was then added to the concentrated starch slurries and the dispersion was gentle agitated for approximately half an hour. The volume of distilled water added was approximately half the volume supernatant previously removed. The washed starch granule slurry was transferred to a sealed container ready for drying. It was not possible to accurately measure the concentration of the slurry prior to drying due to difficulties in taking a representative sample of the rapidly settling starch granule suspension.

The samples of the starch slurry were dried using two different drying systems, spray drying (using the Buchi Mini Spray Drier) and tray drying (using the forced fan, laboratory oven) as described in Section 3.8. The spray dried samples (Samples A, B, C, and D) were preheated to 40 °C (below the gelatinisation point of oat starch [23]) for approximately 30 minutes prior to spray drying. All of the spray dried samples were dried using the same drier operating conditions. The tray dried sample (Sample H) was placed in a flat plastic tray for drying. The tray was left to overnight (approximately 16 hours) in a refrigerator at 4 °C. During this time the starch granules settled to the bottom of the tray. The clear supernatant was carefully decanted off the top of the starch sample and discarded. Table 4.5 summarises the drying conditions used for all of the samples in this trial.

Table 4.5: Drying Conditions for the Oat Starch Slurry

Parameter		**Tray drying**	**Spray drying**
Sample ID		H	A, B, C, D
Inlet air temp	°C	35	140
Outlet air temp	°C	35	80 - 85
Drying time		20 hours	Not measured (seconds)
Final moisture content	%	Not measured	4.37

After drying, the tray dried sample was milled in the Retsch Ultra-Centrifugal Mill and AS 200 Sieve Shaker as described in Section 3.8.

4.3.4. Scanning Electron Microscope Analysis

The structure of the dried starch granules was analysed using a JEOL JSM 7000F field emission gun scanning electron microscope located at the Department of Mechanical Engineering, University of Canterbury [144]. All five oat starch samples were prepared for the scanning electron microscopy using a standard method by an experienced operator, who also operated the equipment. Small amounts of each sample of the dried starch powder were carefully mounted on to aluminium stubs using conductive carbon adhesive tape. These were then sputter coated from a gold leaf source to impart conductivity to the surface of the sample. The thickness of the gold coating was approximately 10 nm. The samples were viewed and images taken at various magnifications, ranging from X500 to X5000. Images were captured of the large scale structure and appearance of each oat starch sample as well as close up observations of the structure and appearance of individual starch granules. Evidence of interactions between starch granules were noted.

4.3.5. Granule Size Measurement

The particle size distribution of the starch granules in an aqueous dispersion was measured using a Mastersizer 2000 Particle Size Analyser as described in Section 3.9.

4.3.6. Composition Analysis

The composition of the extract liquor (clear supernatant) and dried starch samples were analysed to determine any differences in the composition between samples, as described in Section 3.10. It was not possible to analyse the composition of the initial starch slurry as it was difficult to get a uniform sample of this rapidly settling dispersion for analysis.

4.4. Results

4.4.1. Dried Oat Starch Structure

Scanning electron microscope images were taken at various magnifications to investigate the overall and detailed appearance of each sample.

Overall Appearance

Images were taken at lower magnification to provide information about the overall appearance of the dried starch granule samples. Figures 4.2 to 4.6 show scanning electron microscope images at 1000X magnification for Samples A, B, C, D, and H respectively. These images show that the majority of the starch granules present in the spray dried Samples A, C, and D were located in tightly packed agglomerates that were roughly spherical in shape. The scale is shown in the bottom right hand corner of each image.

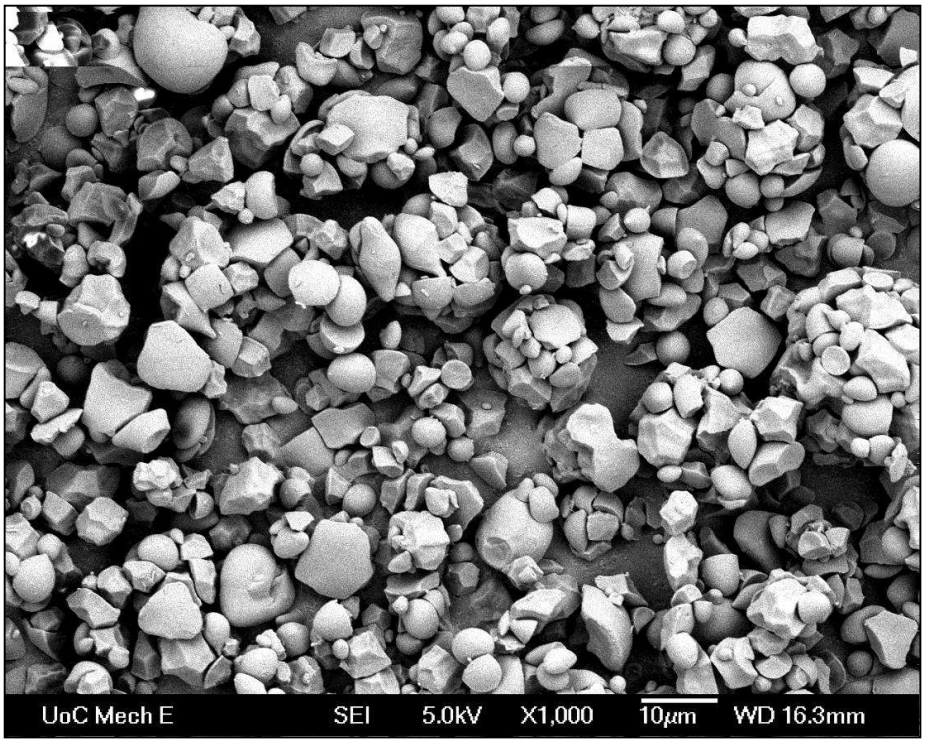


Figure 4.2. The overall appearance of Sample A.

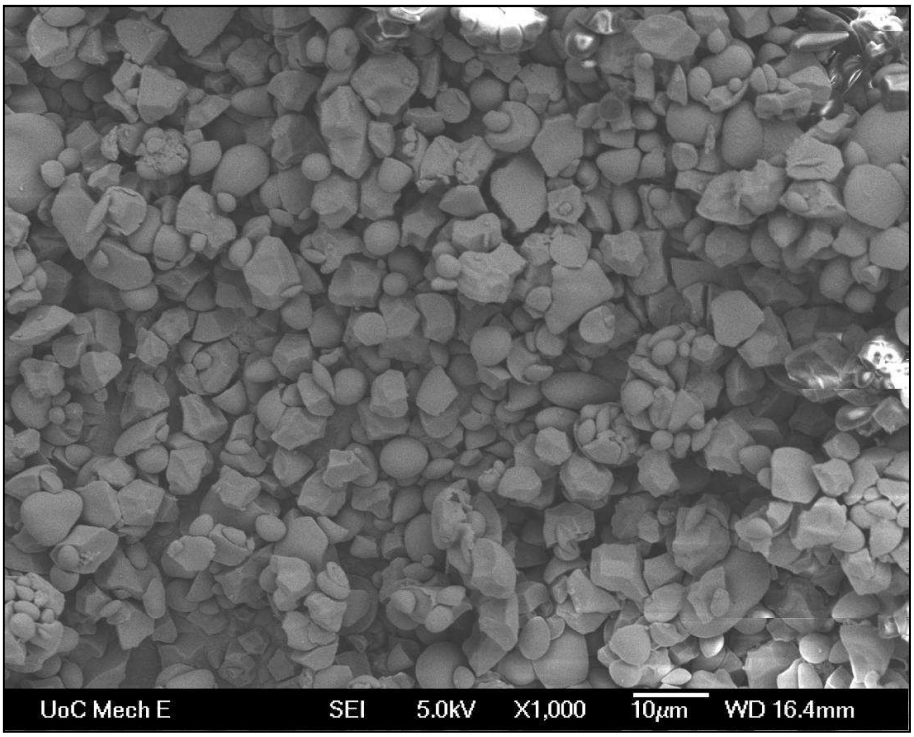


Figure 4.3. The overall appearance of Sample B.

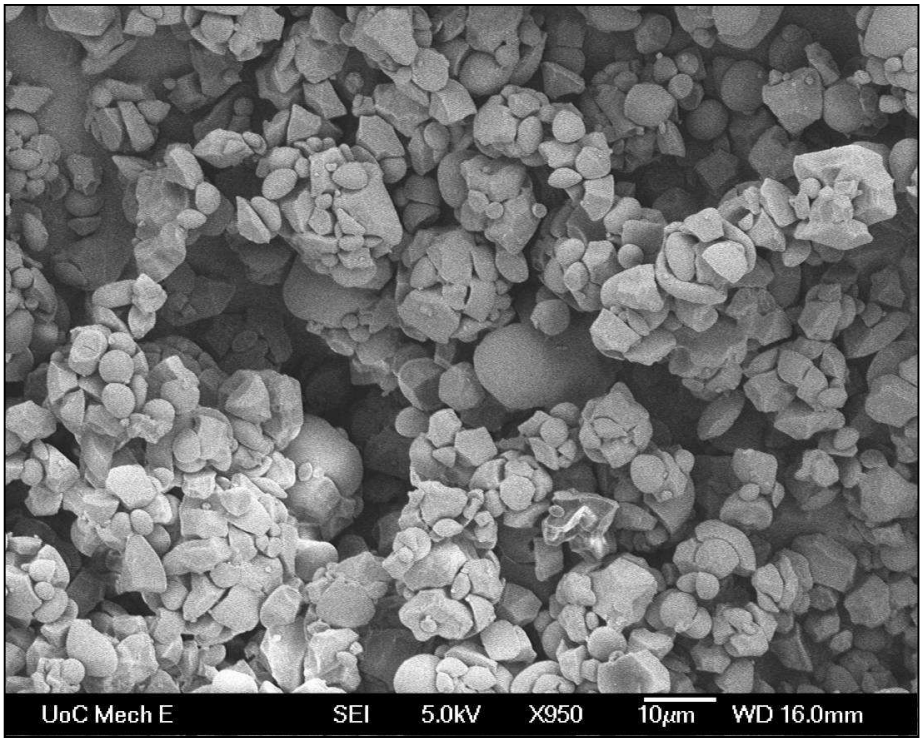


Figure 4.4. The overall appearance of Sample C.

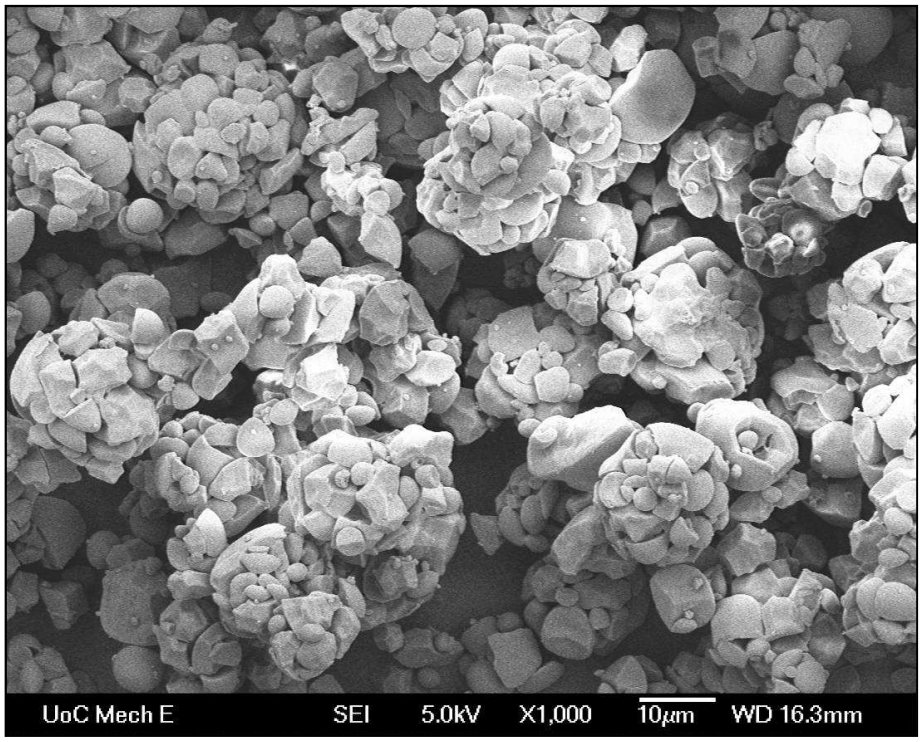


Figure 4.5. The overall appearance of Sample D.

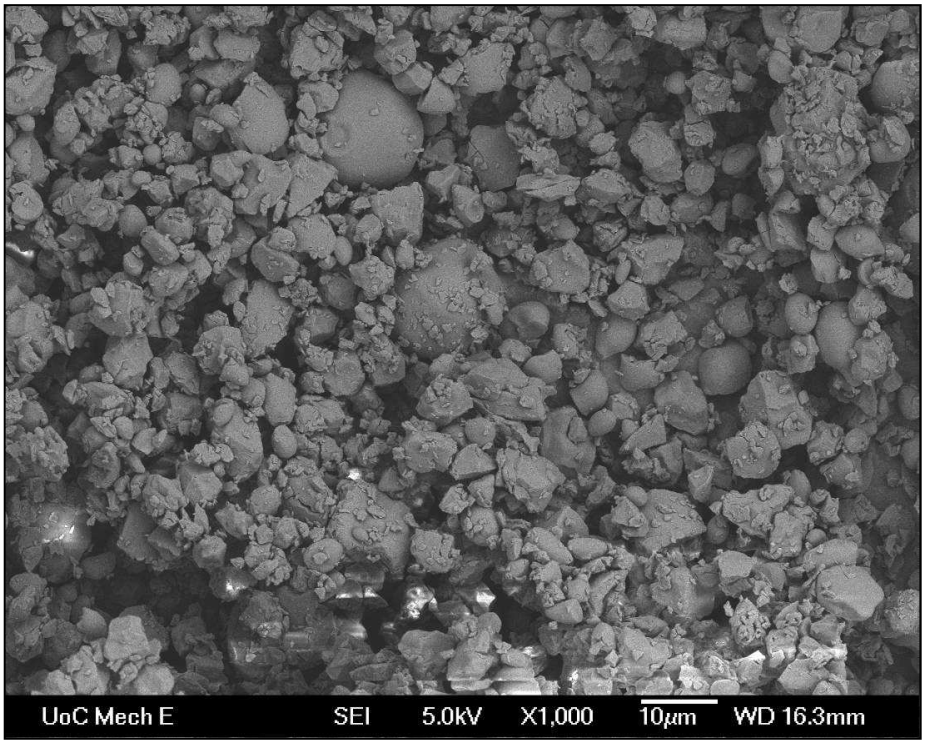


Figure 4.6. The overall appearance of Sample H.

4.4.2. Detailed Appearance

Higher magnification images were used to observe the structure of the dried starch granule samples in detail, with particular focus on the agglomeration of starch granules and the location of any protein particles. Figures 4.7 to 4.11 show detailed scanning electron microscope images at 2500X magnification for Samples A, B, C, D, and H respectively. These images show material bridging the gap between the individual starch granules in Samples A, C, and D. High magnification scanning electron microscope image of Sample C at 5000X magnification is shown in Figure 4.12. This image clearly shows where material bridges the gap between individual starch granules. The scale is shown in the bottom right hand corner of each image.

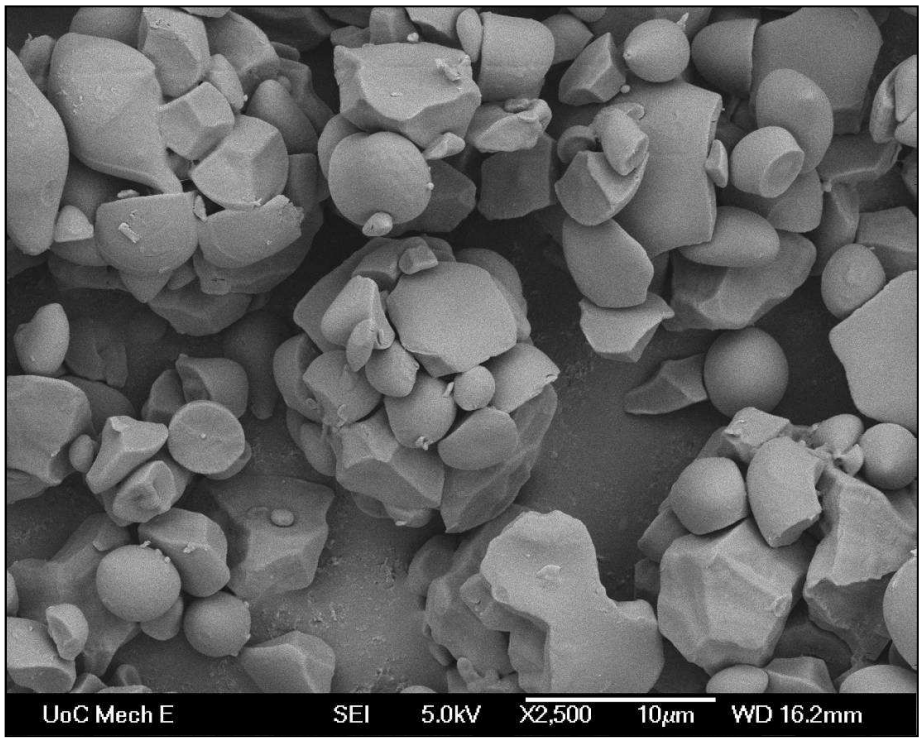


Figure 4.7. The detailed appearance of Sample A.

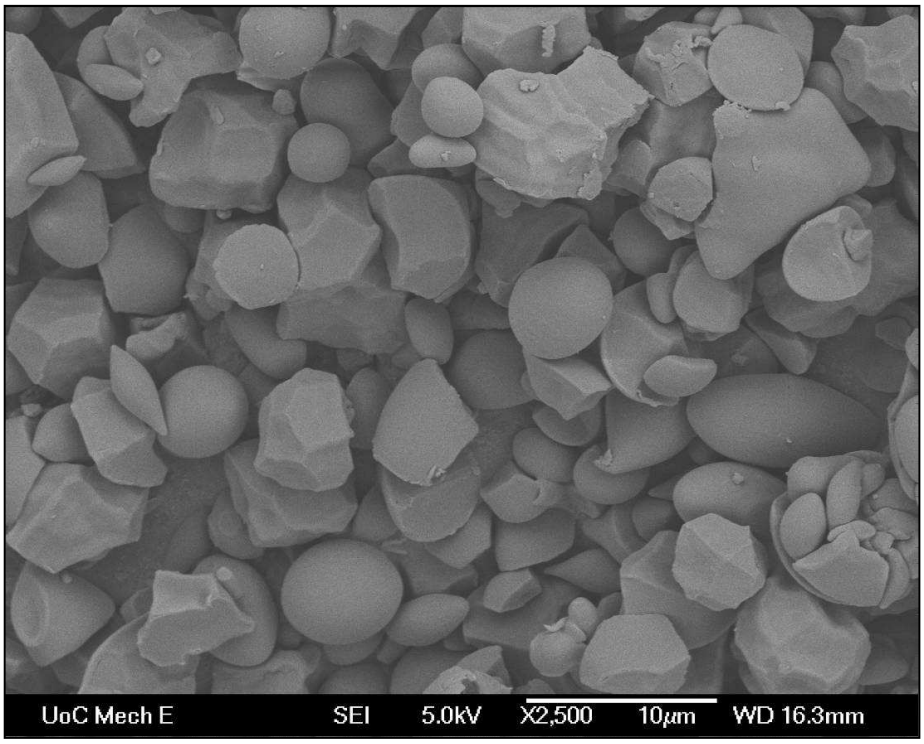


Figure 4.8. The detailed appearance of Sample B.

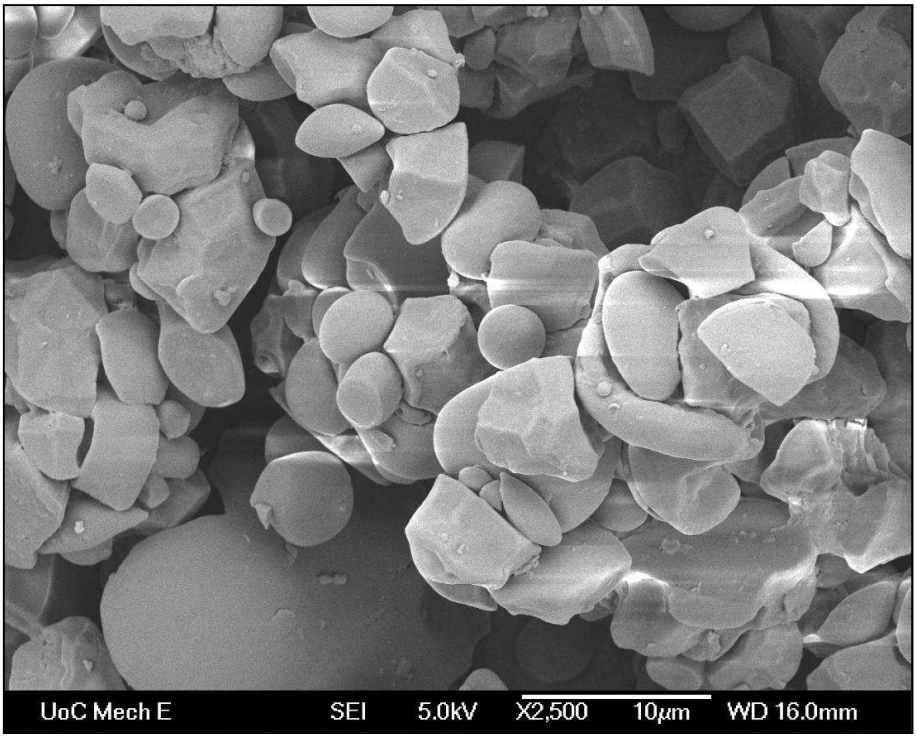


Figure 4.9. The detailed appearance of Sample C.

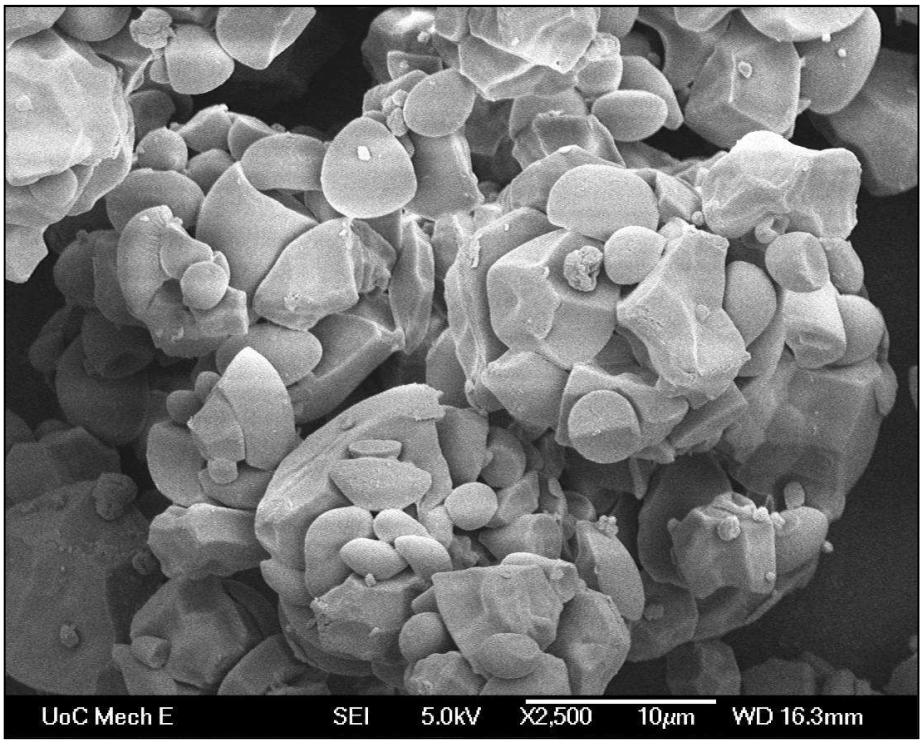


Figure 4.10. The detailed appearance of Sample D.

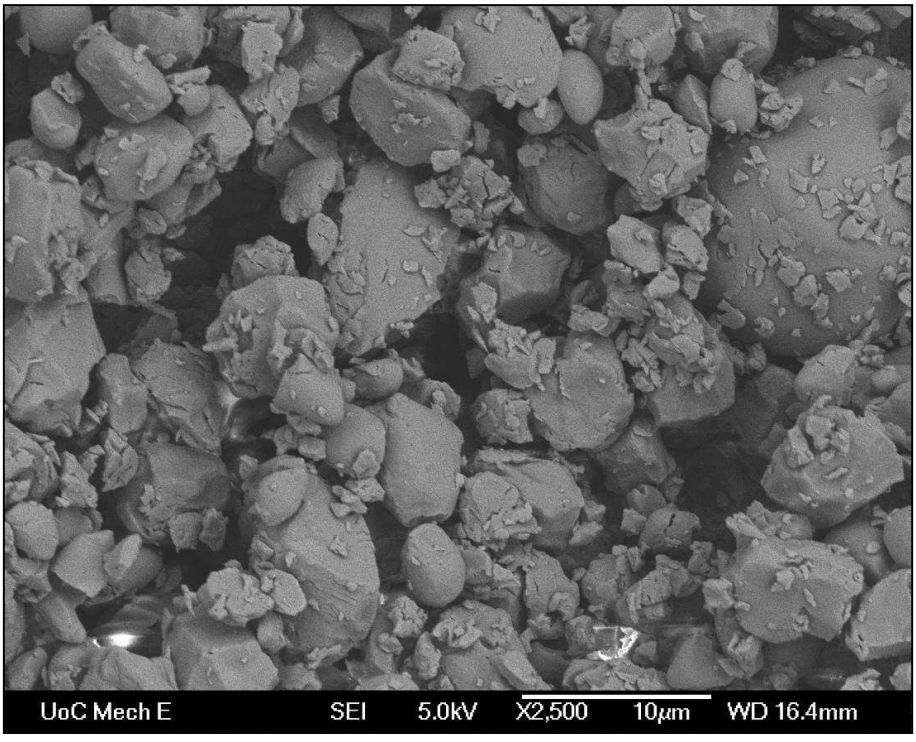


Figure 4.11. The detailed appearance of Sample H.

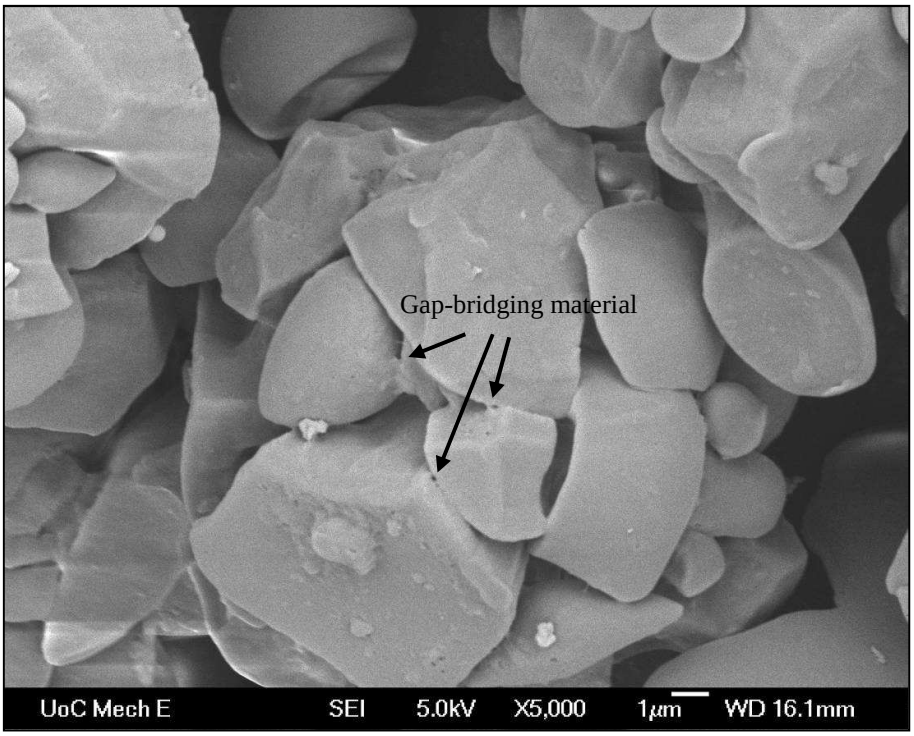


Figure 4.12. Close up image (X5000), showing evidence of bridging between individual oat starch granules in Sample C.

4.4.3. Starch Granule Size Distribution

The Mastersizer provided the derived size distribution data including histograms showing the particle size distribution and statistical analysis of the distribution. Table 4.6 summarises the starch granule size distribution data and shows that the size of the oat starch granules in Samples A and D is larger than Samples B and C. Sample H was not measured, as the Mastersizer method involves dispersing the starch granules in water. The damage starch granules were visible in the scanning election microscope images for Sample H. Damaged starch is known to have increased swelling and solubility in cold water [25, 26]. Hence, measurements using the Mastersizer would not provide accurate measurement of the particle size distribution of this sample. The data output from Mastersizer, including the histograms can be found in Appendix A.

Table 4.6: Oat Starch Size Distribution

Sample	d(0.1) [1] (μm)	d(0.5) [2] (μm)	d(0.9) [3] (μm)	D[3,2] [4] (μm)	D[4,3] [5] (μm)	Span [6]
A	5.08	10.67	22.00	9.05	12.37	1.58
B	4.86	9.12	16.50	8.06	10.02	1.28
C	4.67	9.54	18.450	8.13	10.72	1.45
D	5.33	10.94	21.82	9.34	12.49	1.51

1) d(0.1) is the size of the particle below which 10 % of the sample lies.
2) d(0.5) is the size of the particle below which 50 % of the sample lies, or Mass Mean Diameter (MMD).
3) d(0.9) is the size of the particle below which 90 % of the sample lies.
4) d[3,2] is the Surface Area Mean Diameter (SAMD), or Sauter Mean.
5) d[4,3] is the Volume Mean Diameter (VMD).
6) Span is the measurement of the width of the distribution = [d0.9)-d(0.1)]/d(0.5)

4.4.4. Composition

The composition of two samples of extract liquor (clear supernatant) from the initial starch extraction (from Sample A) and second starch purification (from Samples B and H) were measured and the results are shown in Table 4.7. Both the initial extraction liquor and the purification liquor were very dilute aqueous solutions. The initial extraction liquor contained the soluble biopolymers present in the original dough sample. These were significantly diluted in the starch purification stage.

Table 4.7: Composition of Extract Liquor (mass % on wet basis)

Sample	Total solids (±0.05)	Ash (±0.02)	Protein (±0.05)
First extraction liquor (from Sample A)	0.58	0.05	0.17
Starch purification liquor (from Samples B and H)	0.21	0.01	0.09

The protein content of the dried starch samples was also analysed to identify any differences in the composition between the dried starch samples. These results are shown in Table 4.8. Protein contamination of the starch can come from either protein particle contamination or soluble protein present in the residual extract liquor. Sample C has the highest protein contamination.

Table 4.8: Protein Concentration of the Dried Starch Samples

Sample	**Protein** (%) (±0.1)
Sample A	1.5
Sample B	1.0
Sample C	2.3
Sample D	1.9
Sample H	1.1

4.5. Discussion

4.5.1. Structure Analysis

The scanning electron microscope images show clear differences in the structure of the samples of the starch rich fraction as a result of the prior extraction processes. A detailed, matrix based, comparison between samples is presented in Appendix A.

Protein Particle Contamination

Residual protein particles have been previously reported in scanning electron microscope images as small irregularly shaped particles adhering to the surface of the larger starch granules for wheat starch [133]. All of the samples contained evidence of low levels of residual insoluble protein particles, which were identified by particle morphology. This was expected. These protein particles were observed in the scanning electron microscope images as small irregular shaped particles, usually attached to the surface of individual oat starch granules. These particles were evident in Samples A, B, C, and D. It is not possible to differentiate between small protein particles and fragmented starch granules in Sample H. Three of samples in this study (A, C, and D) were taken without any additional purification to remove residual protein particles. The remaining two samples (B and H) received only a single purification stage. An optimised separation and purification process for the starch would involve several starch purification stages which would be expected to reduce insoluble protein particle contamination of the starch.

Qualitatively, more protein particles were visible in Sample C (Figures 4.4 and 4.9), compared with the other samples. This was consistent with the protein content analysis in Table 4.8, which showed more protein contamination in Sample C (2.3 %) compared to the other samples. Sample C comprised the starch slurry produced by the purification of the protein enriched dough. It was expected that this would contain more protein contamination, as this purification step involved additional mixing and deformation of the protein rich dough. It was likely that small protein particles were broken away from the dough during the extra mixing involved in the protein purification step. These small particles contaminated the starch slurry. In an optimised separation and purification process, contaminated starch from the protein purification stages would be kept separate from starch from the initial extraction.

Using techniques to selectively stain the protein and/or starch combined with standard microscopy techniques or confocal laser scanning microscopy would give a better differentiation of the protein and starch components and the relative of the location of protein particles and starch granules [138]. However, these staining protocols are aqueous based would have destroyed any structures in the dried starch granule powder samples that were unstable in water (i.e. held together by water soluble materials). This was undesirable as the purpose of this study was to observe the dried structure of the starch samples. Hence, selective staining techniques were not used.

Dried Structure

The majority of the starch granules present in the spray dried Samples A, C and D (Figures 4.2, 4.4, and 4.5) were located in tightly packed agglomerates that were roughly spherical in shape. Spray dried particles can form regularly shaped agglomerated structures [145]. This effect has been previously observed by others for fine granule starches such as amaranth, rice, and tapioca [146-149]. There were no obvious agglomerates visible in Sample B, which was also spray dried (Figure 4.3). Most of the starch granules in this sample were present as individual granules. Sample B received a starch purification step to remove the residual extract liquor and replace this with water. The soluble biopolymers present in the original dough (such as soluble proteins, sugars, beta-glucans, and other soluble biopolymers [145]) were

transferred into the extract liquor during the initial extraction. The starch in Samples A, C and D were not purified prior to spray drying. Hence, the residual extract liquor containing the soluble biopolymers was present in the starch slurry of these samples during drying. Table 4.7 confirms that the total solids content of the extract liquor from initial extraction (Samples A, C, and D) was higher (0.59 %) than the supernatant from the starch purification stage (Sample B) (0.21 %). This suggests that the soluble biopolymers present in the initial wash liquor influenced the generation of the oat starch granule agglomerates.

It is proposed that the soluble biopolymers provided a mechanism for the individual starch granules to adhere together during and after the spray drying process. This is supported by the findings of Zhao and Whistler [150] who observed that spray dried amaranth starch slurries with a high soluble protein content produced "popcorn balls" of individual amaranth starch granules which were cemented together. Higher magnification images show material bridging the gap between the individual starch granules in Samples A, C, and D (Figures 4.7, 4.9, and 4.10), which is more pronounced in D. This gap-bridging material is clearly visible in the X5000 image of Sample C (Figure 4.12). This clearly demonstrates that the individual starch granules are physically stuck together. Common naturally derived binding materials in spray drying processes include soluble starches, carbohydrates, sugars, gums and proteins [29]. Soluble biopolymers such as these are present in the oat flour and would have been transferred to the extract liquor during the extraction process. The protein content of Sample B (1.0 %) was lower than the protein content of Samples A, C, and D (see Table 4.8). This supports the earlier finding that the soluble fractions present in the extract liquor are influencing the formation of the roughly spherical oat starch granule agglomerates.

The influence of the soluble biopolymers on the drying process could be investigated by controlling the composition of the extract liquor. A series of experiments could be undertaken that involve dispersing pure starch granules in liquors containing soluble biopolymers. These liquors could be "manufactured" to predetermined biopolymer compositions. Spray drying these slurries would provide information on influence of

different biopolymer components on the morphology of spray dried starch. This was beyond the scope of this research project.

Starch granule agglomerates have not been reported for larger granule starches such as wheat starch and barley starch. Starch granule agglomerates have been reported for smaller granule starches such as amaranth and rice starches [146, 149-151]. This suggests that starch granule size influences the creation of the starch granule agglomerates in the spray drier. This is likely to be due to the smaller starch granules having a greater relative surface area to adhere to other starch granules. The influence of starch granule size on the drying process could be investigated by controlling the size starch granules being spray dried. A series of experiments could be undertaken that involve dispersing starch granules of different size distributions in the same extract liquor and then spray drying. This was beyond the scope of this research project.

These trials have not indentified how the roughly spherical shape of the agglomerates was created. It is likely that this structure is an artefact of slurry droplets formed inside the spray drier. Starch granules are hydrophobic with an outer layer containing both lipids and proteins [24]. During spray drying, the starch granules are likely to migrate towards the outside of the water droplets in order to minimise the surface area in contact with the water. As the droplet dries, the spherical shape of the granules forms. This could be investigated by treating the surface of starch granules to makes them less hydrophobic prior to drying. Another approach could be to replace starch granules in the slurry with different particles of similar size, but with different surface properties and then to compare the resultant dried particles. This was beyond the scope of this research project.

Acidic Conditions

In these trials the most distinct oat starch granule agglomerates were observed in Sample D (Figure 4.5), where acidic conditions were used to enhance the initial separation process [152]. It is reported in literature that acidic conditions hydrolyse starch, altering the functional properties, such as post-gelatinisation viscosity and solubility [26, 153]. Atichokudomchai et al. [148] observed that spherical

agglomerates formed when spray drying acidified tapioca starch. These authors proposed that this was most likely due to partial acid hydrolysis of the starch granule and gelatinisation of the starch granule surface in the spray drier.

Compared to the other samples in this study, the scanning electron microscope images of Sample D show starch granules that appear different, (Figure 4.10). The edges of the granules appear smoother and less angular. This suggests that the acidic conditions of the wash liquor have affected the individual starch granules. It is likely that the acidic conditions caused partial hydrolysis of the oat starch granule surface, which resulted in modification of the functional properties of the starch granules (such as reduced granule hardness and reduced gelatinisation temperature). The altered appearance of the starch granules is mostly likely due this chemical modification of the individual oat starch granules. The more acidic conditions are also likely to have hydrolysed one or more of the soluble components present in the extract liquor. This may have also contributed to the enhanced agglomeration of the individual starch granules.

It is proposed that the acidic conditions caused partial hydrolysis of the surface of the oat starch granules. The surface of the acid modified starch granules was partially gelatinised due to preheating of the starch granule slurry immediately prior to spray drying combined with a reduced gelatinisation temperature of the chemically modified starch granules. This resulted in softer starch granules and stickier starch granule surface which promoted the formation of starch granule agglomerates. This could be investigated by undertaking trials varying the pH of the extract liquor. The results would characterise the influence of extract liquor pH on the formation of the starch agglomerates. Varying the acid used to lower the pH would provide further useful information on the formation of the starch agglomerates. Comparing the viscosity profile of the granules prepared using acidic and standard conditions would identify if the starch had been modified by the acidic conditions. This was beyond the scope of this research project.

Tray Drying

The scanning electron microscope images of Sample H (Figures 4.6 and 4.11) had a different appearance compared to all of the other samples. Sample H was tray dried (not spray dried). The tray dried oat starch slurry formed a cohesive cake that was not readily broken apart. Milling was required to break up the dried starch cake to generate the commercially desirable powder form for the starch. Mechanical processes such as milling are well known to damage starch granules [25, 134]. Damaged starch granules were not desired, as damaged starch granules are known to have altered starch functionality. For example, damaged starch granules have increased cold water solubility [25, 26, 34]. The most obvious feature of Sample H was the presence of a large number of small particles, <1 μm diameter. These were irregular in shape and all had sharp edges, rather than the smoother edges of the oat starch granules observed in the other samples. The majority of these particles were identified as damaged starch granules that had been broken apart in the milling process. Another feature of Sample H was the presence of fracture lines (cracks), gouges, and grooves which were visible on the surface of the larger oat starch granules. This clearly demonstrated that the milling process caused damage to the individual oat starch granules. This was undesirable. However, tray drying produced a starch granule cake that did not readily break apart but formed large chips. Milling was a necessary processing step to generate the desired powder form for the starch. Tray drying and milling was not considered a suitable manufacturing process for oat starch granules from the Al-Hakkak Process.

A few starch granule agglomerates were visible in Sample H and these were irregular in size and shape. This indicates that the spherical shape and uniform size of the starch granule agglomerates in the other samples was due to the spray drying process.

4.5.2. Starch Granule Size

The scanning electron microscope images show that the oat starch granules have an angular appearance and an overall spherical shape. Hence, the assumption used in the Mastersizer calculation that the particles are spherical is considered appropriate.

Literature typically reports particle size distribution for oat starch granules in the range of 2 µm to 15 µm [26, 135, 136, 154]. Mastersizer results (Table 4.6) show the volume mean diameter for the dispersed oat starch granules, for all samples that varied from 10.0 µm (Sample B) to 12.5 µm (Sample D). This is smaller than the oat starch granule agglomerate size for Samples A, C and D observed in the scanning electron microscope images which were typically in the range of 15 µm to 30 µm. This suggests that the oat starch granule agglomerate structure largely disintegrated in the aqueous dispersion and that the starch was predominantly in the form of individual granules. Hence, it was concluded that the agglomerated oat starch granules could not have been present in the original aqueous extract liquor and must have formed during the spray drying process.

Purifying the oat starch slurry by adding water to the separated oat starch granules from the initial extraction (Sample B) resulted in a smaller volume mean diameter of the dispersed oat starch granules (10.0 µm) compared to Samples A and D. A lower d(0.1) and d(0.9) was also observed, as was a reduction in the measured span of the particle size distribution. The starch purification step removed most of the soluble material present in the initial extract liquor. The scanning electron microscope images show that Sample B did not form starch granule agglomerates during spray drying. Combining this result with the volume mean diameter results for Samples A, B and D, this suggests the agglomerated structure present in Samples A and D did not fully disintegrate in the aqueous dispersion. It is concluded that some starch granule agglomerates remained in the dispersion, which resulted in a larger measured volume mean diameter for Samples A and D compared with Sample B.

The volume mean diameter of the oat starch dispersion was similar for Samples A (12.4 µm) and D (12.5 µm). Both samples received single extraction starch step. Sample D was settled in acidic conditions. This suggests that the acidic extraction conditions did not significantly affect the size or solubility of the oat starch granules in the aqueous dispersion and did not alter the disintegration of the dried oat starch granule agglomerates in the aqueous dispersion.

The protein purification (Sample C) resulted in a smaller volume mean diameter of the dispersed oat starch granules (10.7 µm). The scanning electron microscope images for Sample C showed that agglomerates were present in the spray dried oat starch powder. The initial extraction process removed granules that were not tightly trapped in the protein network. The protein purification stage removed the granules that were more tightly trapped within the protein network and required more processing to extract. This result suggests that the more tightly trapped granules released in the second wash were smaller.

4.6.Conclusions

Scanning electron microscopy provided valuable information on the influence of extraction and drying processes on the structure of agglomerates of the dried oat starch granules, extracted using the Al-Hakkak Process. The scanning electron microscopy images identified differences in the structure and location of the oat starch granules and residual protein particles as a result of extraction, purification and drying. This research confirmed the hypothesis that drying conditions can be controlled to produce a fine powder of individual, undamaged, oat starch granules. This research has shown that the extraction, separation and purification conditions as well as the drying process influence the structure of the spray dried oat starch granule agglomerates, disproving the second hypothesis for this trial.

The research shows that the presence of soluble material in the extract liquor produced roughly spherical agglomerates when the oat starch slurry was spray dried. It was concluded that this was due to the soluble biopolymers from the oat-gluten flour being transferred to the extract liquor and bridging the gap between starch granules. These biopolymers acted as an adhesive and glued individual starch granules together to form the agglomerates. This study has identified that acidification of the extract liquor enhanced this agglomeration. It was concluded that this was due to increased stickiness of the granule surface brought about by the partial acid hydrolysis of the

starch granule, which reduced the gelatinisation temperature and created a stickier granule surface during the spray drying.

It was concluded from the Mastersizer results that the oat starch granule agglomerate structure present in three of the samples investigated largely disintegrated in the aqueous dispersion. However, the structure did not fully disintegrate into individual granules during the measurement period. Acidic conditions did not significantly affect the size or solubility of the oat starch granules and did not alter the disintegration of the dried oat starch granule agglomerates in the aqueous dispersion. It was concluded that the more tightly trapped oat starch granules released in the second wash were smaller that the less tightly bound granules separated in the initial extraction.

Milling of the dried starch cake following tray drying caused undesirable damage to the individual oat starch granules. Individual oat starch granules were gouged, fractured, and broken into smaller pieces. Hence, it was concluded that tray drying and milling was not a suitable manufacturing process for oat starch granules from the Al-Hakkak Process.

In a commercial process (such as those discussed in Section 2.9.2 of this thesis), purification of the starch would be required to remove the low levels of residual insoluble protein particles that were visible in all of the spray dried the samples. Thus, the final product would be likely to be most similar to Sample B (Figure 4.3) unless the composition of the starch slurry was controlled to ensure it contained soluble biopolymers. Protein contamination was exacerbated by collecting the starch from the protein purification. These starch granules also had a smaller diameter. It is recommended that this starch is not combined with the starch from the initial extract liquor in a commercial manufacturing process.

5. Oat-Gluten Dough Rheology

5.1.Introduction

This chapter discusses rheology trials carried out on sheeted oat-gluten dough prepared using a blend of oat and gluten flours.

In 1929, E. C. Bingham first described rheology by the generally accepted definition: "*the study of the deformation and flow of matter*" [155]. Rheology involves the measurement of the physical response of a material to an external mechanical force. Rheology has been widely applied by scientists and engineers in the study of various materials, such as rubber, plastics, paints, and various biopolymers [155]. Since dough manufacturing involves applying mechanical force during kneading, rheology measurements have been used in the study of dough. The rheology of dough has been widely analysed and reported in literature, for example: Kokelaar; Dobraszczyk and Morgenstern; van Vliet, Stojceska; and Bulter et al. [156-158]. These studies, and numerous others, have correlated dough rheology measurements to various baking parameters such as dough sheeting performance and bread making performance. Dobraszczyk and Morgenstern [156] recently carried out a comprehensive review of dough rheology in relation to bread-making.

It was considered that rheology measurements would provide useful information that could be correlated with the performance of the oat-gluten dough in the Al-Hakkak Process [51, 52]. Little has been published relating dough rheology to the separation of starch granules and gluten proteins from wheat dough. No previous studies have investigated the rheology of oat-gluten dough produced using a blend of oat flour and wheat gluten flour. No previous studies have correlated oat-gluten dough rheology with the separation of oat starch from oat-gluten protein using the Al-Hakkak Process [51, 52].

The hypothesis for these trials was that changes (either chemical or physical) occur in the protein network of the sheeted oat-gluten dough during the resting period that follows kneading and rolling/sheeting. It was proposed that the processing and/or

composition of the oat-gluten dough influence these rheological changes and that large deformation rheology can be used to measure this.

The aim of this work was to assess the effect of varying processing conditions (such as composition, kneading, and resting) on the large scale deformation characteristics of sheeted oat-gluten dough and relate this to starch and protein separation efficiency. The focus of the trials undertaken and hence, the information reported here, was to observe if variations in the processing conditions resulted in measurable differences in the rheology during the resting period that follows kneading and rolling/sheeting. The desired outcome was to calculate fundamental rheological parameters including stress, deformation (elongation), deformation rate, and elongation viscosity of oat-gluten dough samples that could be correlated to other process performance data such as yields and purity.

5.1.1. Rheology – An Introduction

Materials are generally divided into three groups based on rheological properties; elastic, viscous, and visco-elastic [157]. Dough is a visco-elastic material, which means it displays both elastic and viscous characteristics.

An elastic material can deform and store all of the strain energy when a stress is applied. The strain energy is completely released when the stress is removed and the material returns to its original conformation. When a deformation is applied to an ideal viscous material, the material flows and all of the strain energy is dissipated as heat. The material does not return to its original shape when the stress is removed. The rheology theory for materials with elastic and viscous properties, such as dough, is well established and documented in literature and a summary follows [157, 159].

Stress (σ) is function of the force applied (F) and the cross sectional area (A) over which the force is applied.

$$\sigma = \frac{F}{A} \qquad 1$$

Depending on the direction of the deformation (parallel or normal to the area), the ratio of the stress and the strain (e) is termed either: the shear modulus (G), or Youngs (tensile) Modulus (E). Under linear elastic conditions the following relationship holds:

$$\sigma = Ge \quad 2$$

For viscous materials the ratio between the stress and relative strain rate ($\dot{e}$) is termed the viscosity (η).

$$\sigma = \eta\dot{e} \quad 3$$

For visco-elastic materials the situation is more complex as the relationship between the stress and strain depends on the time scale of the deformation. Often at very short timescales the material behaves elastically with viscous characteristics becoming more apparent as the timescale grows [157]. This means that the stress that is required to maintain a specific strain decreases with increasing timescale. Also, part of the deformation is permanent and this increases over time. In addition it follows that the value of the modulus reduces with longer timescales. Many authors have shown that dough displays visco-elastic characteristics for example: Dobraszczyk and Morgenstern; Bloksma and Bushuk; and Morgenstern et al. [156, 160, 161].

The characteristic time of a visco-elastic material (τ) can be calculated for various rheological properties relative to the flow time [162]. For dough, characteristic resting time is a measure of how rapidly stresses retained in the dough as a result of kneading reduce (usually termed "dough relaxation") during a resting period (t_R) relative to the rheological parameter of interest. Characteristic resting time may vary depending on the rheological parameter being examined (strain hardening index, strength index and apparent modulus of activity) and can be calculated using Equation 4, where A and B are constants and X is the rheological parameter of interest. As $\tau \rightarrow 0$, the material behaves more elastically and as $\tau \rightarrow \infty$ the material displays increasingly viscous rheology.

$$X = A + Be^{t_R/\tau} \quad 4$$

5.1.2. Dough Rheology

As discussed, rheological measurements can provide valuable information on the characteristics of visco-elastic materials such as dough. This information is produced by measuring the forces required to deform the material using various mechanisms such as compression, extension, and shear. Dobraszczyk and Morgenstern [156] summarise the common rheological measurement techniques used to characterise wheat dough and relate these to manufacturing processes.

Rheology can be useful to assist with process development by providing information on key fundamental characteristics of the material, such as stress, strain, strain hardening, and viscosity. It can also provide valuable information about the failure of materials when force is applied. However, it is important that the method of rheological measurement selected is representative of the manufacturing process being investigated [156]. The scale of the measurements must be appropriate and the applicability of the results to the processes involved should be considered.

It is widely reported that wheat dough rheology displays visco-elastic properties [107, 157, 161, 163]. This means that the scale (both spatial and time) of the rheological measurements undertaken on dough can have a significant effect on the results. Due to the difficulties in measuring the visco-elastic rheology of dough, empirical techniques have been developed and widely used by industry and researchers. The key techniques are well summarised in the review by Dobraszczyk and Morgenstern [156]. However, these empirical techniques do not provide fundamental rheological measurements and standard fundamental rheological parameters cannot be determined from the data these techniques produce. The results, although repeatable, vary depending on the equipment and operating conditions used (for example temperature and strain rate) [156, 161, 164-166].

5.1.3. Dough Rheology Measurement Techniques

It is reported in literature that measurement of large and small scale deformations produce different rheological results [156, 161, 165, 167]. Long chain, high molecular mass (HMW) biopolymers such as gluten, can produce different rheological results depending on the scale of the rheological test being undertaken [156]. Also, it is important to undertake rheological measurements of dough under appropriate deformation conditions for the processes being investigated. This means that to obtain useful information, it is vital to select the appropriate test method and test conditions for the material and process being investigated.

The most common fundamental rheological test methods used for dough include [156, 157, 165, 168]:

- Small deformation dynamic shear oscillation.
- Small and large deformation shear and creep tests.
- Large deformation measurements.
- Flow viscometry.

Measurements of fundamental rheological properties using small deformation oscillatory, shear and creep methods have been widely used and there is a well developed theoretical background. Fundamental rheological data can be produced such as storage modulus, phase angle, viscosity, stress, and strain. However, these tests are limited to small strains, essentially linear shear and high extension rates. Dough kneading imparts large strains, multidirectional shear and comparatively smaller extension rates. Hence, the results from these methods are not considered representative of the large scale deformation processes that take place during kneading [156, 161].

Large deformation techniques are carried out at a scale that is similar to the processes applied to dough (both spatial and time scales). The data produced by large deformation methods is representative of the kneading and extraction processes used in the Al-Hakkak Process and could provide useful and relevant information. However,

large deformation methods are not as rigorous as the well-documented, small scale, oscillatory shear methods. Limited rheological parameters can be calculated from the force and elongation data produced by large deformation data. Parameters that can be calculated include; stress, strain, strain rate, strength index (*K*), and stain hardening (*n*). It was considered important to measure the rheology that is representative of the processes involved and these rheological measurements were considered sufficient for this study. Thus, large deformation rheology was selected for this investigation.

There are three main options for large deformation measurements:

a) **Uniaxial extension**, where wheat dough (usually a narrow strip) is stretched in one direction, as described in detail by Keiffer et al. [169]. This is usually done by clamping both ends and then using a hook to pull the dough upwards from the middle. The force and extension are measured and rheological parameters calculated from this data. The main problem reported with this method is premature failure close to the end grips. Also, this method measures extension in only one direction. The method is the complex and time consuming set up for equipment and samples. For these reasons this method was not selected for these trials.

b) **Biaxial compression and extension**, where wheat dough (usually a short cylinder) is compressed and allowed to expand in two perpendicular directions, following the method described in detail by Kokelaar [157]. The dough cylinder is placed between two lubricated surfaces (to minimise friction) and the plates are compressed. The force and extension are measured and rheological parameters calculated from this data. This method was not selected, due to the problems with friction between the dough and the plates affecting the results. The test is also time consuming and difficult to set up.

c) **Biaxial extension**, where a wheat dough sheet is stretched in two perpendicular directions. Examples are described in literature [161, 170, 171]. In the method by Morgenstern et al. [161], a dough sheet is deformed axisymmetrically as described later in this chapter (Section 5.2.1) and rheological parameters calculated from the data. The dough rheology is determined by a power law relationship between stress and strain, with strain hardening effects also

calculated. The main advantage of this method is that is quick and simple to set up and measurements can be taken very quickly following dough production (sheeting). This allows the change in rheological parameters during the resting period immediately following dough making to be measured. This method was selected for this investigation as the changes in rheology of the oat-gluten dough throughout the resting period was of interest.

5.2. Methodology

The oat-gluten dough samples in these trials were prepared following the initial stages of the Al-Hakkak Process as shown in Figure 5.1 [51, 52]. Samples of sheeted oat-gluten dough were taken immediately following the kneading and sheeting processes. Large deformation rheology measurements were undertaken following the method described in detail by Morgenstern et al. [161]. The production of the sheeted dough samples including the kneading, sheeting, and resting processes are described in detail later in this chapter (Section 5.2.3).

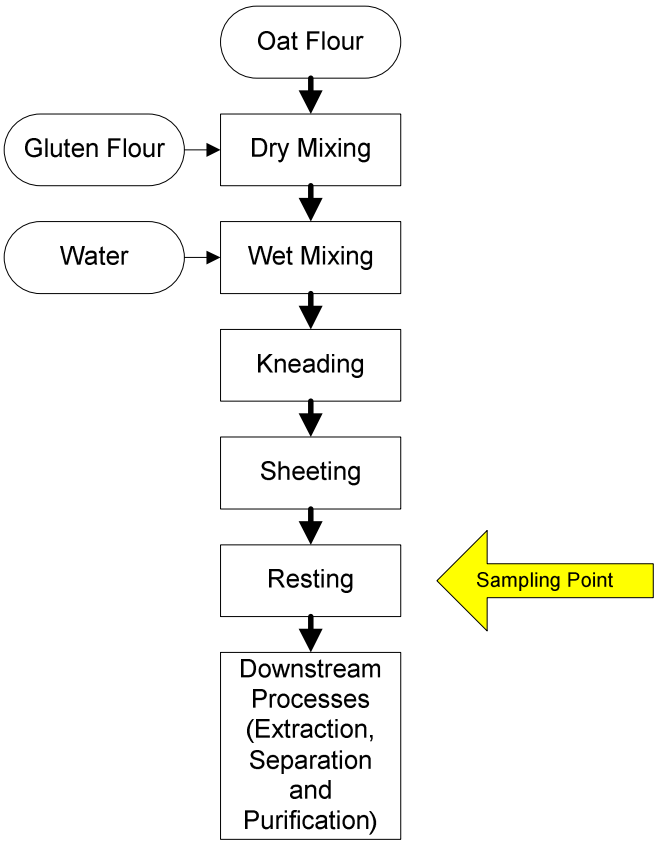


Figure 5.1. Schematic diagram showing the sampling point in the Al-Hakkak Process for these trials.

5.2.1. Dough Rheology Measurement

Rheology measurements were performed using a Universal Testing Machine (Model 1011 Instron, Canton, MA) (Instron). The Instron was set up as described in detail by Morgenstern et al. [161] and as shown in Figures 5.2 and 5.3. In summary, the sheet deforming device consisted of two square acrylic plates with aligned 55 mm (±0.01 mm) apertures with a slightly rounded edge (radius approximately 1 mm) and a stainless steel probe of 35 mm (±0.01 mm) diameter with slightly rounded edge (radius approximately 1 mm). Disks of sheeted oat-gluten dough samples were placed in-between these two acrylic sheets, centred over the 55 mm diameter apertures. An approximately 95 mm diameter ring of pins were located in each acrylic plate, concentric with the 55 mm apertures. These pins were 1.2 mm diameter and protruded approximately 2 mm above the plate surface. The purpose of these pins was to penetrate the dough sheet to secure it into position and reduce the risk of slippage. The

sheet deforming device was mounted on the Instron and lined up so that the plate holes and the probe were centred vertically (axially symmetrical).

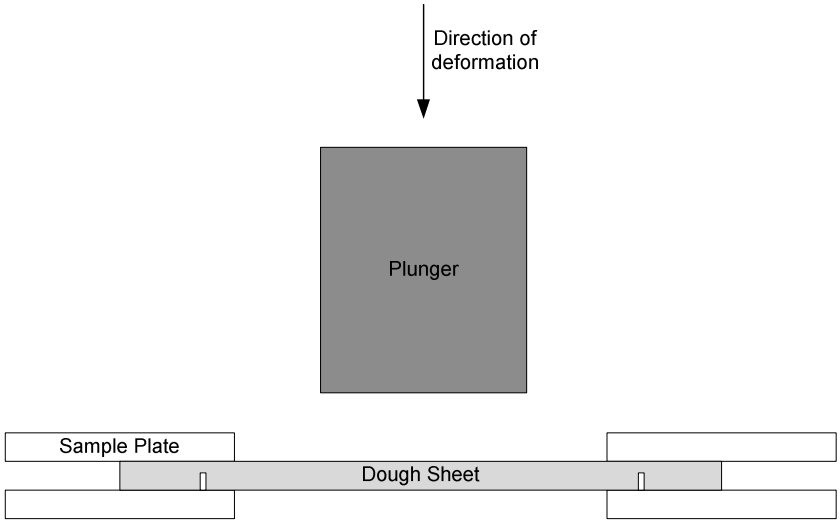


Figure 5.2. Schematic elevation view of the experimental set up.

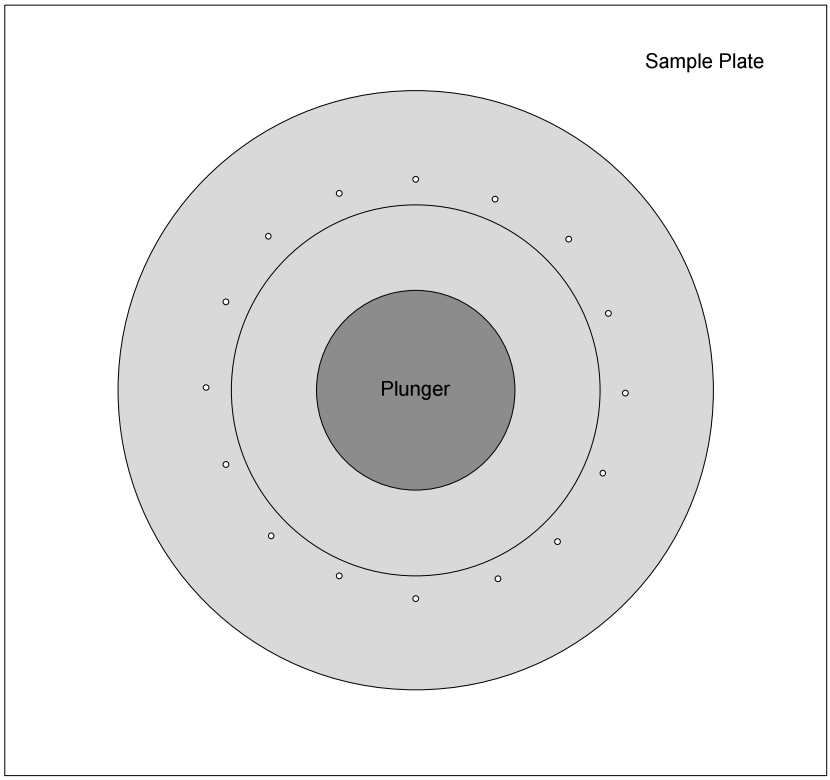


Figure 5.3. Schematic plan view of the experimental set up.

Stress and Strain Calculation

During operation, the probe moved down through the aperture in the plate at a predetermined speed and extended the disk of oat-gluten dough to form a flat topped cone. Extension of the dough sheet occurred in the 10 mm aperture between the edge of the plate apertures. Previous work by Morgenstern et al. [161] had confirmed that there was very little slippage/flow of the dough across the surface of the probe and the effect of this was negligible.

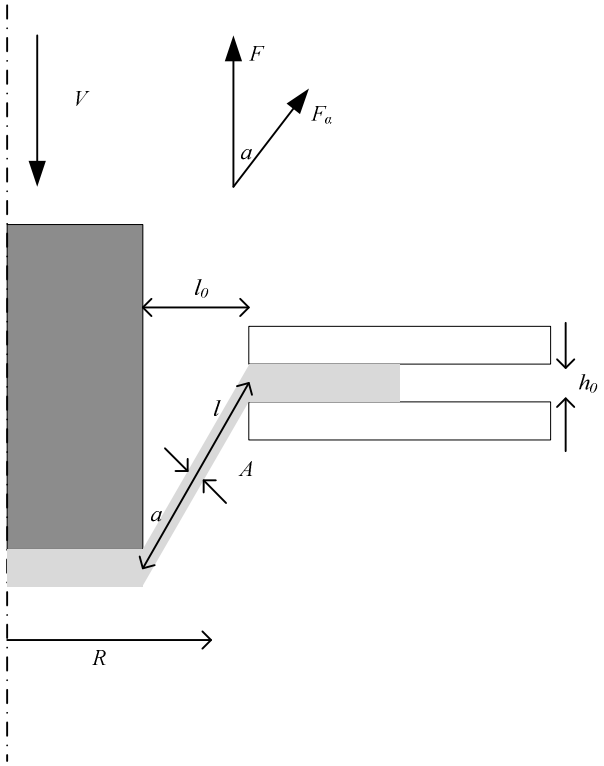


Figure 5.4. Nomenclature used for large deformation calculations, summarised from Morgenstern et al. [161].

For Figure 5.4:

- l_0 is the initial gap between the circumferences edges of the plunger and the aperture (10 mm)
- l is the length of the extended dough sheet between the plunger contact edge and the edge of the aperture

- A is the average cross sectional area of the dough sheet between the plunger centre-line and the edge of the aperture
- R is the average radius between the plunger and the edge of the aperture (65 mm)
- h_0 is the initial thickness of the dough sheet
- F is the force on the probe
- F_s is the force component in the direction of the sheet
- v is the velocity of the plunger.

The calculation of the average stress from this experimental set up is given in detail by Morgenstern et al. [161] and a summary of this calculation is given below.

At any given time l is:

$$l(t) = \sqrt{l_0^2 + v^2 t^2} \quad (5)$$

The elongation (strain) rate can be calculated as follows:

$$\dot{e}(\hat{t}) = \frac{1}{l}\frac{dl}{dt} = \frac{1}{t_c}\frac{\hat{t}}{1+\hat{t}^2} \quad (6)$$

Integration yields:

$$e(\hat{t}) = \frac{1}{2} ln(1+\hat{t}^2) \quad (7)$$

where:

- t is the time period
- t_c is the characteristic time for the flow (deformation), $t_c = {}^{l_0}/_{v}$
- $\hat{t}$ is the normalised time, $\hat{t} = {}^{t}/_{t_c}$

The stress at a function of time can be calculated from F_s and A as follows:

$$\sigma(t) = \frac{F_s(t)}{A(t)} \quad (8)$$

The average cross sectional area (*A*) is:

$$A(\hat{t}).l(\hat{t}) = 2\pi l_0 R h_0 = constant \quad 9$$

Substituting for *l(t)*with 1 gives:

$$A(\hat{t}) = 2\pi R h_0 \, {}^{1}\Big/\sqrt{1+\hat{t}^2} \quad 10$$

It follows that the average stress can be calculated using the following relationship:

$$\sigma(t) = \frac{F(t)}{2\pi R h_0}\hat{t} \quad 11$$

The forces and extension data from the Instron were captured using in-house software, which allows the operator to set specific measurement parameters, such as cross head speed (extension velocity), dough thickness, zero position, probe diameter, and hole diameter. The equations presented above were used to calculate specific rheological parameters (stress, strain, and strain rate) based on the captured data as well as the operator inputted information.

Strain Hardening Calculation

The phenomenon of strain hardening is widely reported for wheat dough and a recent publications by van Vliet [80, 172] summarise the current knowledge. It is understood that the gluten in wheat dough provides the strain hardening properties [172]. Hence, strain hardening characteristics are of interest for oat-gluten dough. Stain hardening of visco-elastic materials is most simply described by Hollomon's equation a power law relationship between stress and stain [173].

$$\sigma = Ke^n \quad 12$$

where *K* is the strength index and *n* is stain hardening index (a material property). Hence, strain hardening can be expected to be influenced by variations in material composition and processing. The yield stress (σ_y) of the material can be included in the calculation by applying Ludwick's equation [173]. The yield stress is the point at which plastic deformation starts.

$$\sigma = \sigma_y + Ke^n \quad (13)$$

If the yield stress is very small it can be assumed to be negligible. For dough, the yield stress is very small, typically so small that it is difficult to detect from the stress/strain data. Hence, it can be expected that that the simpler Holloman's equation can be applied to dough. This assumption was tested and confirmed for oat-gluten dough by reviewing the stress/strain plots from these investigations.

Figure 5.5 shows a typical stress strain curves described by Hollomon's equation. Strain hardening occurs where $n > 1$. There is no strain hardening when $n = 1$.

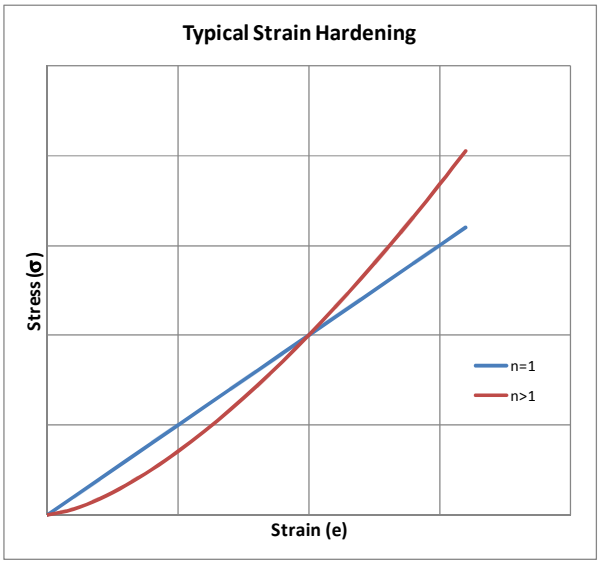


Figure 5.5. Effect of strain hardening shown on a typical stress strain curve.

The strain hardening index can be calculated from the slope of the log(σ) versus log(e) plot. Hence, the rate of strain hardening can be calculated for any specific stress and strain.

$$n = \frac{d\ log(\sigma)}{d\ log(e)} = \frac{e}{\sigma}\frac{d\sigma}{de} \quad (14)$$

$$\frac{d\sigma}{de} = n\frac{\sigma}{e} \quad (15)$$

In the plastic region of the stress-strain curve (stress values higher than the yield stress), the strain hardening index cannot be considered in isolation. It must be considered in conjunction with the strength index as varying strain hardening will alter the strength index for any given stress.

Apparent Modulus of Elasticity Calculation

For large deformation measurements the modulus of elasticity (*E*) can be used as a measure of the strength of interactions between the molecules of the material being measured with stiffer materials having higher elastic modulus values [174]. In the elastic region of the stress-strain curve (stress values lower than the yield stress), the modulus of elasticity remains constant with increasing stress and Hooke's Law can be applied [159]:

$$E = \frac{\sigma}{e} \tag{16}$$

In the plastic region of the stress-strain curve the modulus of elasticity changes with changing stress. In this case an apparent modulus of elasticity (E_a) can be calculated for specific stress values and used to estimate the rheological parameters of the material being tested. A graphical representation of the apparent modulus of elasticity is shown in Figure 5.6.

$$E_a = \frac{\sigma}{e} \tag{17}$$

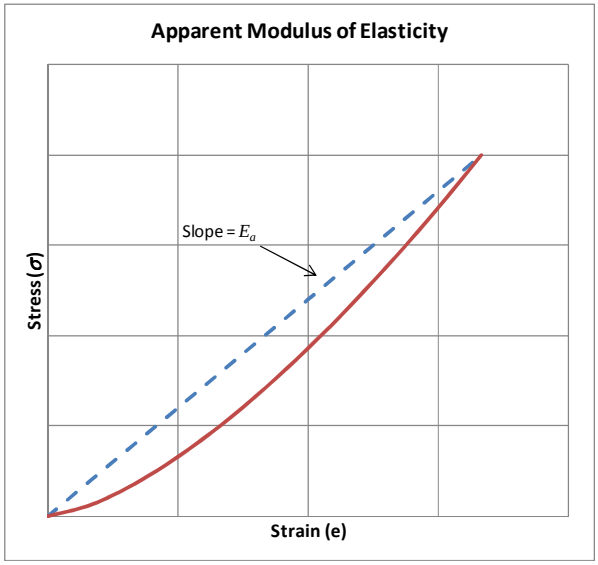


Figure 5.6. Apparent modulus of elasticity (Ea) for a specific stress and strain shown on a typical stress-strain curve.

5.2.2. Equipment Selection

Two different processing scales were investigated. Small pilot scale processing was used as this allowed several small samples to be prepared with a variety of composition and kneading conditions. A single larger pilot scale processing trial provided a uniform oat-gluten dough substrate which was used to produce a set of uniform samples for investigating the rheology changes during the resting period. The small pilot scale dry mixing, wet mixing, and kneading was carried out using a Farinograph mixer fitted with a 50 g kneading vessel. The larger pilot scale dry mixing, wet mixing, and kneading was carried out using 5kg Hobart dough mixer fitted with a 5 kg capacity vessel and a single dough hook ("E" dough arm). Both the small and larger scale processing equipment are described in more detail in Section 3.2.

5.2.3. Sample Specification

The effect of varying several composition and processing conditions were investigated. Seven composition and processing variables were investigated:

1. Salt content of the oat-gluten dough.
2. Gluten content of the oat-gluten dough.
3. Oat flour particle size.
4. Kneading time.
5. Single and double sheeting.
6. Processing scale (small and larger pilot scale).
7. Resting time of the oat-gluten dough.

The influence of resting time on the rheological characteristics of the sheeted oat-gluten dough was investigated with samples prepared using the larger pilot scale Hobart mixer. This provided a larger quantity of uniform material for the resting time measurements. This also allowed direct comparison between the small and larger pilot scale processing. The Farinograph mixer was used to prepare all of the smaller scale samples for investigating the other composition and processing variables. Table 5.1 provides a matrix description of the samples prepared for the small scale trials using the Farinograph to investigate composition and kneading effects. The positive numbers indicate a condition tested at “higher” than the standard, whilst the negative numbers indicates a condition tested at “lower” that the standard. Zero designates a standard setting was used. Samples were prepared in duplicate.

Table 5.1: Experimental Plan for Composition and Kneading Time Samples

Sample	Salt	Gluten	Particle size	Kneading time	Sheeting	Equipment scale
1	0	0	0	0	0	0
3	0	0	0	-1	0	0
2	0	0	0	+1	0	0
12	0	0	0	+2	0	0
4	-1	0	0	0	0	0
5	-2	0	0	0	0	0
6	0	0	0	0	+1	0
7	0	-1	0	0	0	0
8	0	+1	0	0	0	0
11	0	0	-1	0	0	0
10	0	0	+1	0	0	0
PS	0	0	0	0	0	*

* Eleven samples were prepared from a single batch of dough. Each had a different resting time which varied from 2 minutes to 90 minutes.

5.2.4. Sample Preparation

Flour Preparation

Preparation and storage of the oat and gluten flour used in these trials is described in Section 3.3, with two exceptions. Most of the samples were prepared from a single batch of oat flour and gluten flour that was sieved through a 500 μm sieve, to remove bran and any large particles. Samples 10 and 11 were made using the same gluten flour as the other samples, but used oat flour with a different particle size specification.

- Standard flour specification used flour that passed through 500 μm vibrating sieve.
- Sample 10 had a large particle size specification, with the flour having passed through a 720 μm vibrating sieve.
- Sample 11 had a small particle size specification, with the flour having passed through a 250 μm vibrating sieve.

Kneading and Resting

The small pilot scale, Farinograph samples were produced with an initial charge of 48.10 g oat flour as shown in Table 5.2. The larger pilot scale, Hobart mixer, samples were prepared using an initial charge of 480.6 g, as shown in Table 5.3.

Table 5.2: Oat-Gluten Dough Recipe for Composition and Kneading Time Samples

Sample Description		**Standard**	**Low salt** (NaCl) (0.01 %)	**No salt** (NaCl) (0.0 %)	**Low gluten**	**High gluten**
Sample		*	4	5	7	8
Oat flour	g	48.1	48.1	48.1	51.0	45.0
Gluten flour	g	11.9	11.9	11.9	9.0	15.0
2 % salt solution	g	2.0	1.0	0.0	2.0	2.0
Water	g	38.6	39.6	40.6	38.6	38.6
Water temperature	°C	30	30	30	30	30

* All other samples (1 – 3, 6, 10 – 12), except resting time.

Table 5.3: Oat-Gluten Dough Recipe for Resting Time Samples

Sample description		**Pilot scale**
Sample		PS
Oat flour	g	480.6
Gluten flour	g	119.4
2 % salt solution	g	20.1
Water	g	386.4
Water temperature	°C	30

The operating conditions used in the Al-Hakkak Process to produce oat-gluten dough varied between samples and between equipment. Tables 5.4 and 5.5 show the operating conditions used to produce the oat-gluten dough.

Table 5.4: Small Scale Oat-Gluten Dough Processing Conditions

Sample description		Standard	Short kneading	Long kneading	Very long kneading
Sample		*	3	2	12
Kneading temperature	°C	30	30	30	30
Kneading Time [a, b]	seconds	120	90	150	180
Resting temperature	°C	22	22	22	22
Resting time	minutes	90	90	90	90

* All other samples (1, 4 – 11), except resting time samples

a) Other research has identified that 120 seconds kneading in the small scale Farinograph is equivalent to ten minutes kneading in the pilot scale Hobart mixer.

b) Excludes 30 seconds dry mixing prior to water and salt solution addition.

Table 5.5: Larger Pilot Scale Oat-Gluten Dough Processing Conditions

Sample description		Pilot scale
Sample		PS
Kneading temperature	°C	30
Kneading Time [a, b]	minutes	10
Resting temperature	°C	22
Resting time	minutes	0 to 90

a) Excludes 30 seconds dry mixing prior to water and salt solution addition.

b) The Hobart mixer (AE200) used in this trial has three speed settings. The slow setting (43rpm) was used for the first four minutes to allow the flour and water to form a cohesive oat-gluten dough. Then the second setting (150 rpm) was used for the remaining six minutes mixing time. The third setting was not used.

Sheeting

Sheeting was carried out in two stages following the general method described in Section 3.5.

- **Stage 1 sheeting:** Immediately following kneading the oat-gluten dough was placed on a work bench between 22 mm deep guides and manually rolled to a thickness of 22 mm using a stainless steel rolling pin. The dough sheet was rotated 90°. The 22 mm guides were replaced with 10 mm guides and the manual rolling was repeated.
- **Stage 2 sheeting:** The oat-gluten dough sheet was then carefully fed into the dough sheeter, with the rollers set at the maximum 5 mm apart. The rollers

were reset to 3 mm apart and the oat-gluten dough was carefully fed into the dough sheeter again, this time rotated 90°. Sample 6, received a "higher" level of sheeting compared to the standard sheeting. This sample was passed though the dough sheeter twice at the 5 mm and 3 mm settings. For each second pass the dough sheet was rotated 90°.

Each of the small pilot scale samples were sheeted individually, immediately following kneading. The larger pilot scale oat-gluten dough was divided into two parts of approximately equal mass prior to sheeting for easier handling during sheeting.

Disks of oat-gluten dough, approximately 105 mm diameter, were cut from the sheeted dough using a circular, stainless steel, biscuit cutter. These disks were carefully transferred to plastic bags for the resting period. Care was taken when handling the samples to minimise any disturbance of the oat-gluten dough. The plastic bags were sealed to minimise moisture loss from the dough during resting and left undisturbed for the duration of the resting period at a temperature of 23 °C.

Universal Testing Machine (Instron) Measurement

The rheology measurement was undertaken following the method described by Morgenstern et al. [161]. The oat-gluten dough disks were carefully removed from the plastic bags and mounted horizontally on top of the lower of the two acrylic sheets. The upper acrylic sheet was placed on top of the sample. The thickness of the dough and acrylic sheets was measured manually, using digital callipers, and this information recorded. The thickness of the acrylic sheets was accurately known, so the thickness of the dough sheet could be readily calculated. The zero point was visually identified as the surface of the dough and this information was recorded. A constant crosshead speed setting of 50 mm/min was selected and the Instron commenced force and distance/extension measurements using automated software. Measurements automatically stopped when breakage was detected by the Instron (by a rapid reduction in the force being measured).

5.3. Results

5.3.1. Force and Extension

A large amount of force and distance/extension data was collected by the Instron. A graphical summary of this data is presented in Appendix B. All of the sheeted oat-gluten dough samples displayed similar curves, with the force initially increasing with increasing extension up to a maximum. After the maximum the force decreased rapidly with increasing extension indicating that the failure point had been reached. The graph comparing force and extension for the samples produced using standard operating conditions is shown in Figure 5.7 and for all of the resting time samples in Figure 5.8. Graphs of the samples processed with varying composition and processing conditions are shown in Figure 5.9 (a to f). These results are typical of the results for all samples. The maximum force and the corresponding extension differed between samples.

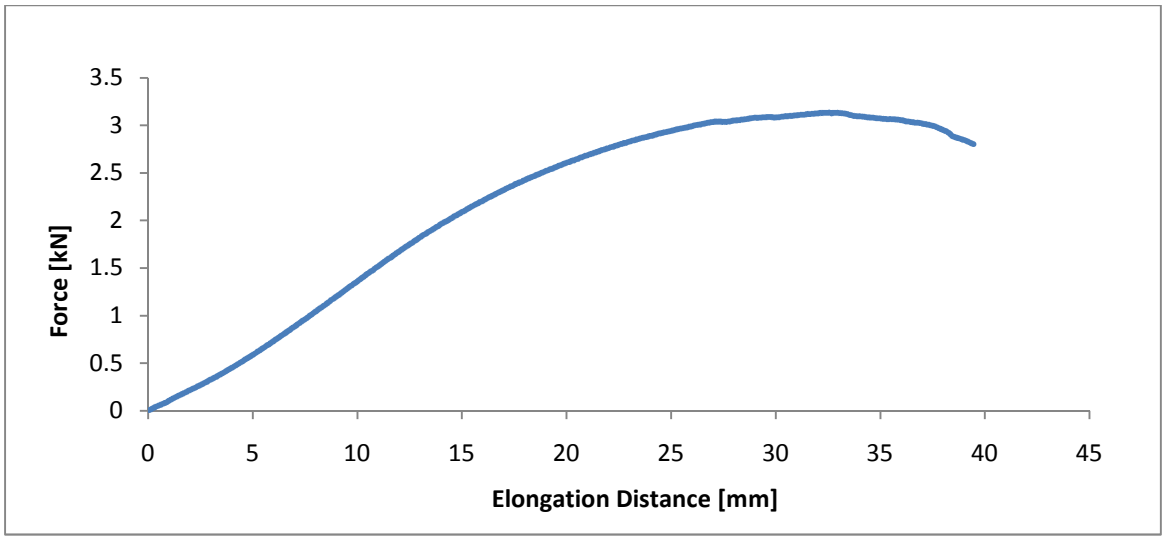


Figure 5.7. Force versus extension plot for samples produced using standard conditions at small pilot scale.

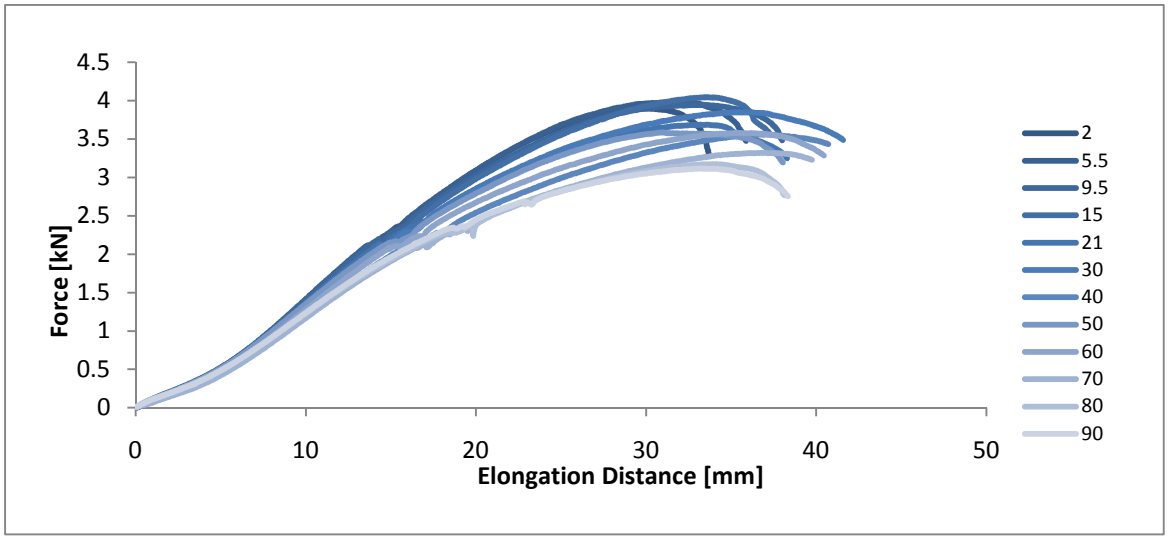


Figure 5.8. Force versus extension plots for samples produced using standard conditions with different resting times (in minutes).

Due to the nature of scale up trials, it was not possible to take a large number of identical samples for analysis. Thus it was not possible to assess the errors between using comprehensive statistical techniques such as standard deviation. Errors were assessed qualitatively by comparing differences between similarly processed samples and observing behavioural trends in the results common to several samples. Only small differences were observed when similarly processed samples were compared. For example, the peak force differed by 1.7% between the samples with a resting time of 80 and 90 minutes and the corresponding elongation differed by 2.5%. Common behaviour trends were observed, for example as the resting time increases that maximum force decreases. These observations suggest that the results are repeatable and thus the measurement error is small. These and other observations are discussed in more detail in the discussion section of this thesis.

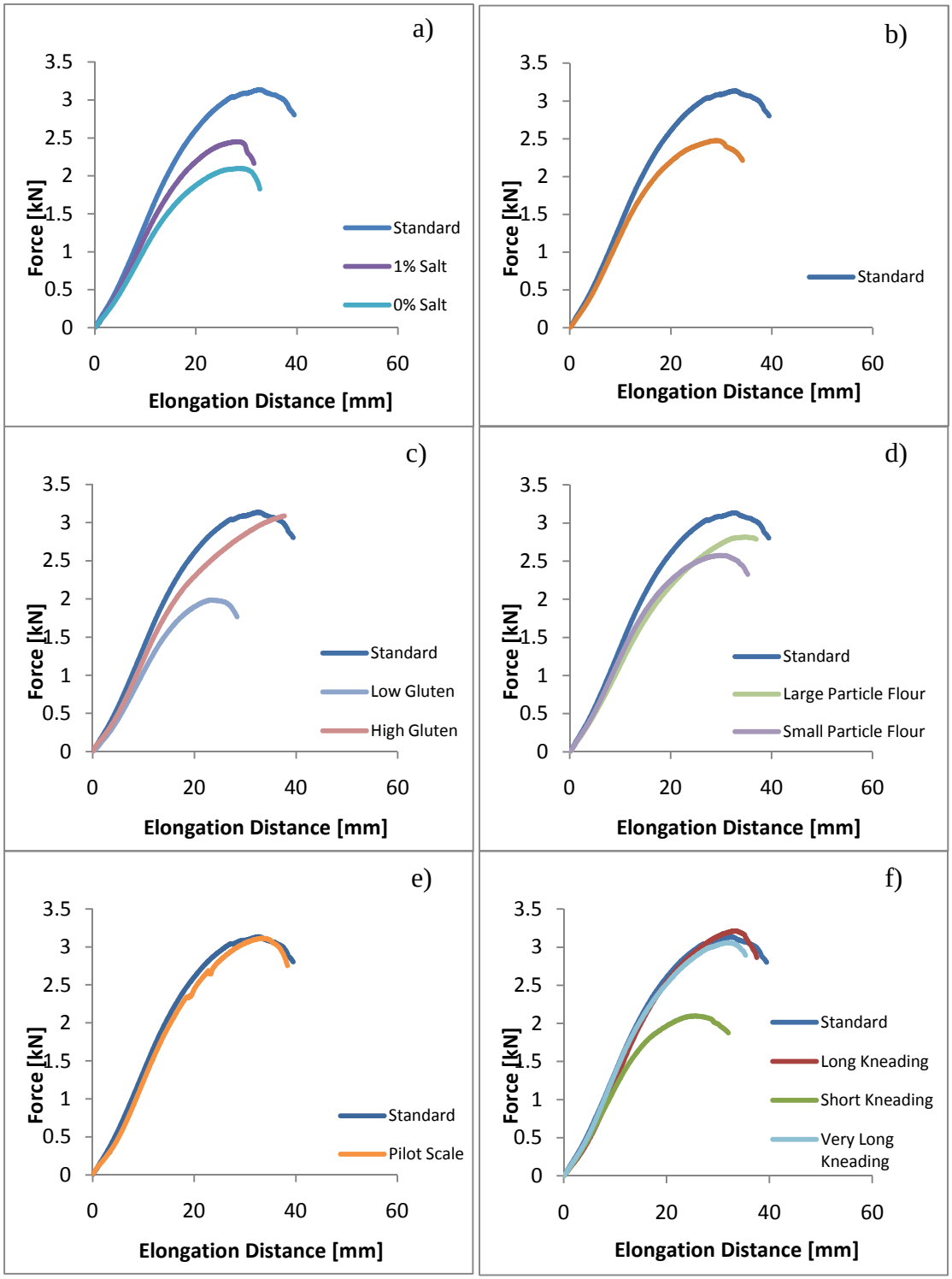


Figure 5.9 (a to f). Force and elongation data for samples processed with varying composition and processing conditions.

5.3.2. Stress and Strain

The rheological properties of individual sheeted oat-gluten dough samples were calculated from the fundamental measurements of force and elongation and a summary of this data can be found in Appendix B.

The stress and strain were calculated for all data points collected for all samples. However, only data for selected points are presented graphically (strain = 0.35, 0.50, 0.75, 1.00, 1.25, and 1.50) as this provides a good representation of the overall data set. These data points were selected as they are often reported in literature so are useful for comparison with other studies [161].

All of the sheeted oat-gluten dough samples showed similar stress versus strain curves, with the strain increasing with increasing stress, although the slope of the curve differed between samples. The curves were concave upwards indicating that stress was increasing more rapidly than strain. This was typical of the all of the samples. The stress versus strain curve for the standard operating condition sample (small pilot scale) is shown in Figure 5.10.

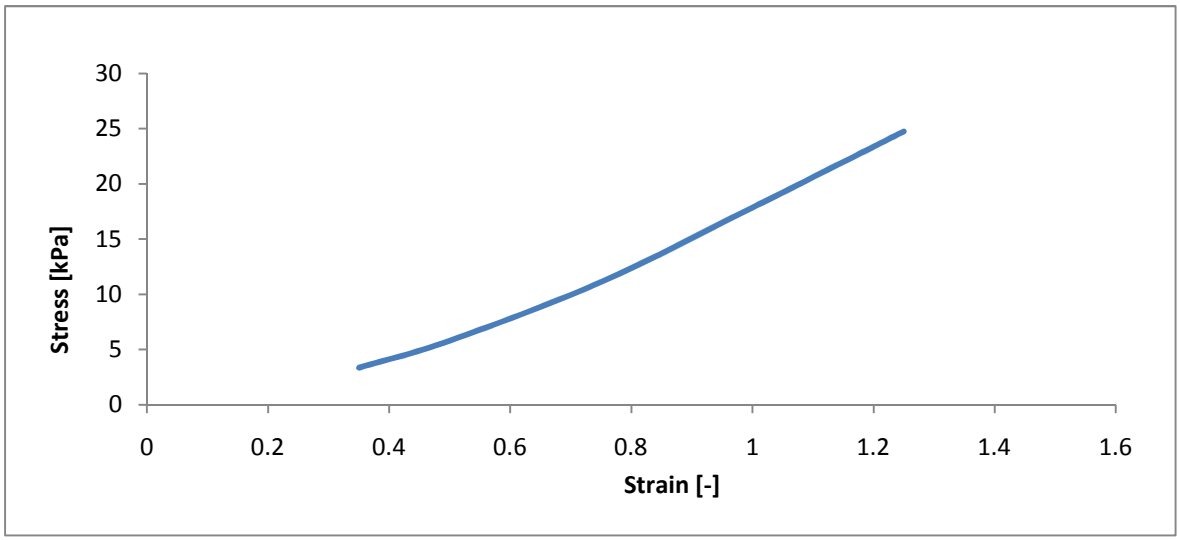


Figure 5.10. Stress versus strain curve for the samples produced using standard operating conditions at small pilot scale.

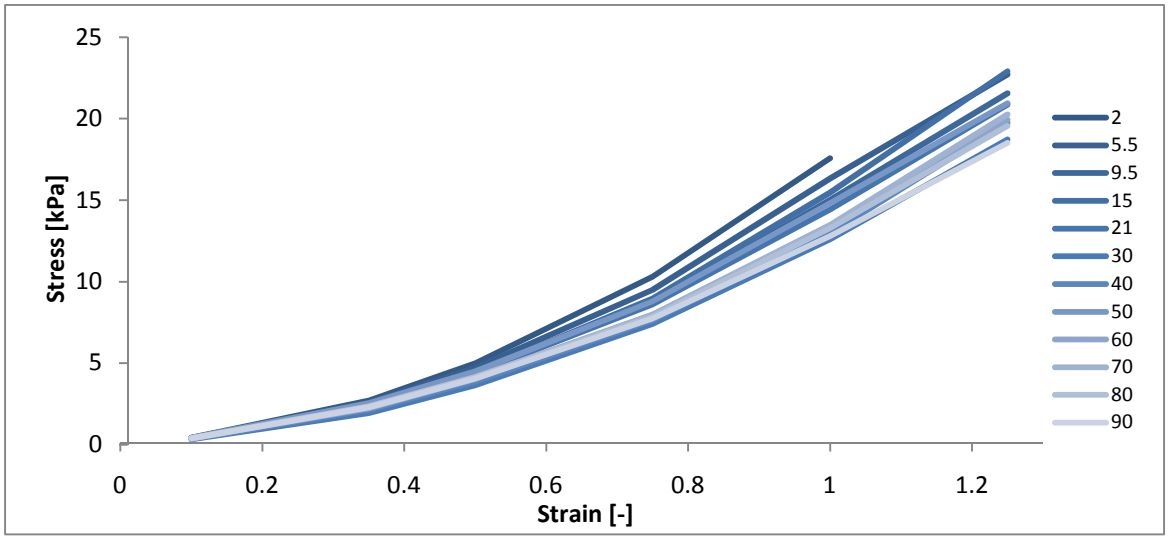


Figure 5.11. Stress versus strain curves for samples processed using standard conditions with different resting times (in minutes).

Changing the resting time produced stress versus strain graphs that were similar in shape with curves that were concave upwards (Figure 5.11). Similarly varying the composition and kneading produced stress versus strain graphs that were similar in shape with curves that were concave upwards (Figure 5.12 a to f). This indicates that strain hardening was occurring in all of the oat-gluten dough samples.

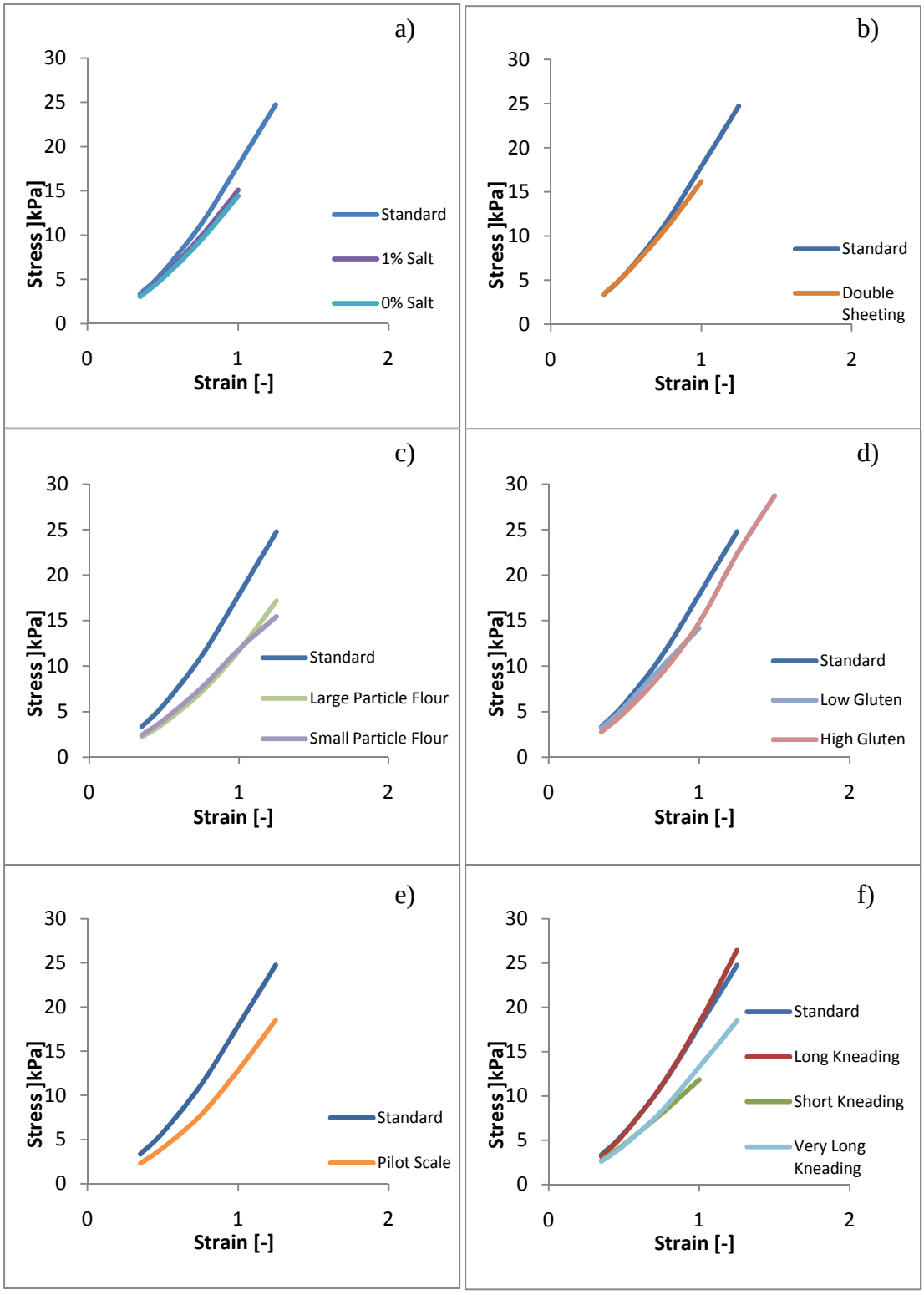


Figure 5.12 (a to f). Stress versus strain curves for samples processed varying composition and processing.

5.3.3. Other Rheological Characteristics

The rheological parameters: strain hardening index (n), strength index (K) and apparent modulus of elasticity (E_a) were calculated from the stress and strain data. Appendix B contains a summary of the strain hardening, strength index, and apparent modulus of elasticity data. For visco-elastic materials such as dough, these rheological parameters are not linear and change with changing stress and/or strain. The situation where strain equals one is often used for comparison between results [175]. Figures 5.13 to 5.15 summarise strain hardening, strength index, and apparent modulus of elasticity for samples processed using standard conditions with different resting times for the situation where strain equals one.

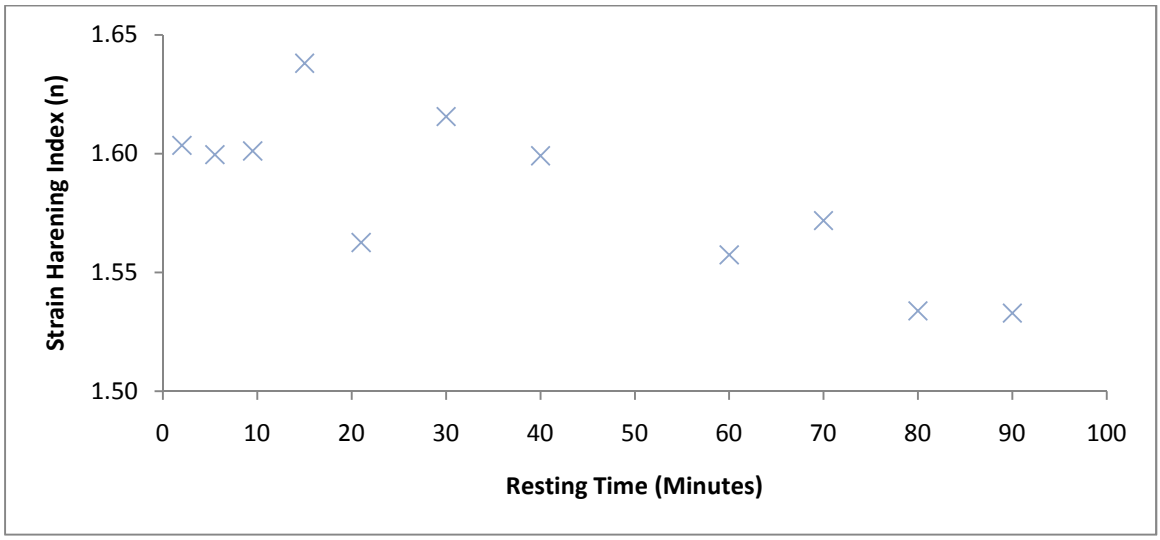


Figure 5.13. Strain hardening for samples processed using standard conditions with different resting times for the situation where *e* = 1.

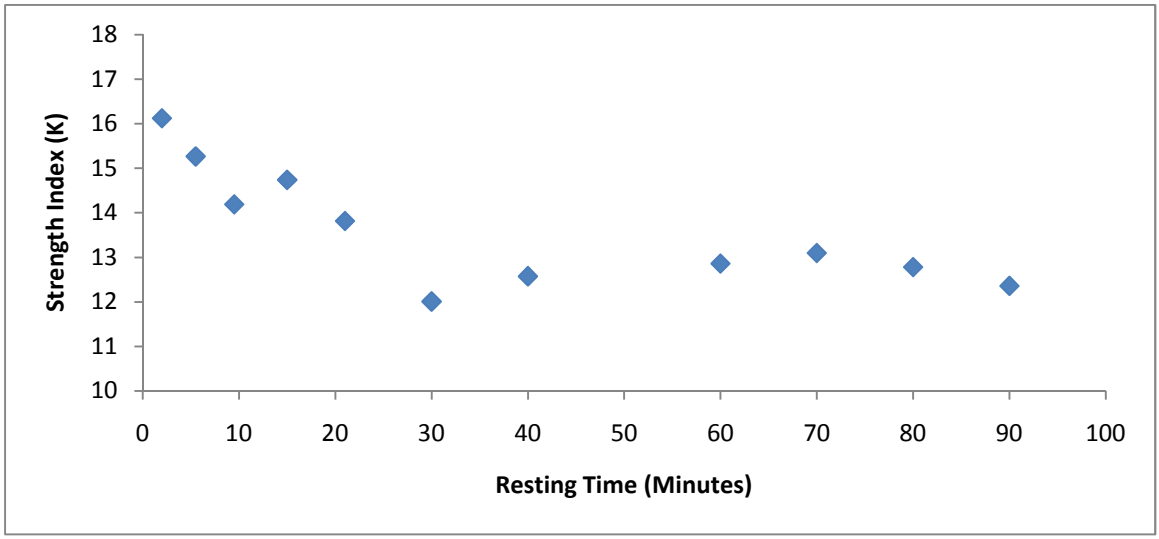


Figure 5.14. Strength index for samples processed using standard conditions with different resting times for the situation where *e* = 1.

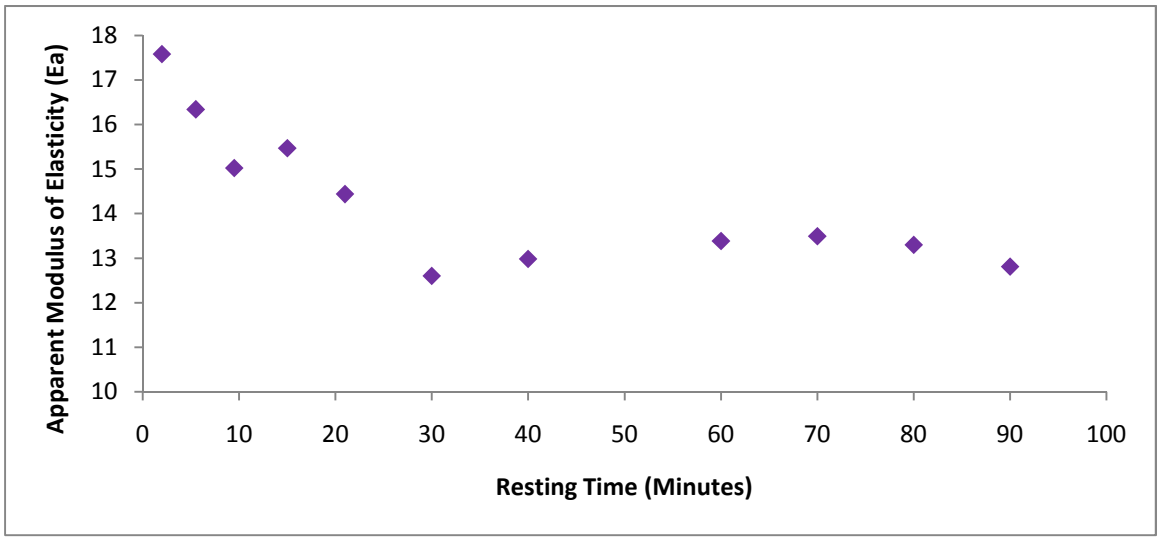


Figure 5.15. Apparent modulus of elasticity for samples processed using standard conditions with different resting times for the situation where *e* = 1.

Table 5.6 summarises strain hardening, strength index, and apparent modulus of elasticity for samples processed varying the composition and processing conditions for the situation where strain equals one.

Table 5.6: Rheology Parameters With Varying Composition and Processing Conditions (for the situation where $e = 1$)

Sample	Parameter	Strength index (K)	Strain hardening index (n)	Apparent modulus of elasticity (E_a)
1	Standard	17.6	1.58	17.9
3	Short kneading	11.9	1.41	13.3
2	Long kneading	18.2	1.67	18.3
12	Very long kneading	13.1	1.55	11.8
4	Low salt (0.01 %)	15.0	1.49	15.1
5	No salt (0.0 %)	14.4	1.48	14.4
6	Double rolling	16.2	1.49	16.2
7	Low gluten	14.5	1.42	14.2
8	High gluten	15.0	1.62	14.8
11	Small particle flour	11.5	1.47	11.7
10	Large particle flour	11.7	1.62	11.9

For the samples with different resting times, a characteristic resting time of the dough (τ) was calculated using Equation 4 for the strength index and the apparent modulus of elasticity for the situation where strain equals one. The characteristic resting time was found to equal 14.7 minutes, and 13.4 minutes for strength index and apparent modulus of elasticity respectively. The characteristic resting time provides a quantitative measure of the time taken for the dough to relax. The characteristic resting time can be calculated from different rheological parameters which can give different results. For these trials the characteristic time was calculated for strain hardening and apparent modulus of elasticity (Figures 5.14 and 5.15). The characteristic resting time was not calculated for strain hardening, due to the large errors associated with this rheological parameter which would make the result meaningless.

5.3.4. Rheology at Sample Failure

The rheology measurement method used in these trials was a destructive technique that stretches individual sheeted oat-gluten dough samples until rupture (sample failure). The rheological conditions at the point where the sample fails is often used for comparison of visco-elastic materials such as wheat dough [175]. The failure point of

each sample was recorded and the stress, strain, strain rate, and apparent modulus of elasticity were calculated. Appendix B contains a summary of this data. Figures 5.16 to 5.18 summarise stress, strain, strain rate, and apparent modulus of elasticity at the sample failure point for samples processed using standard conditions with different resting times.

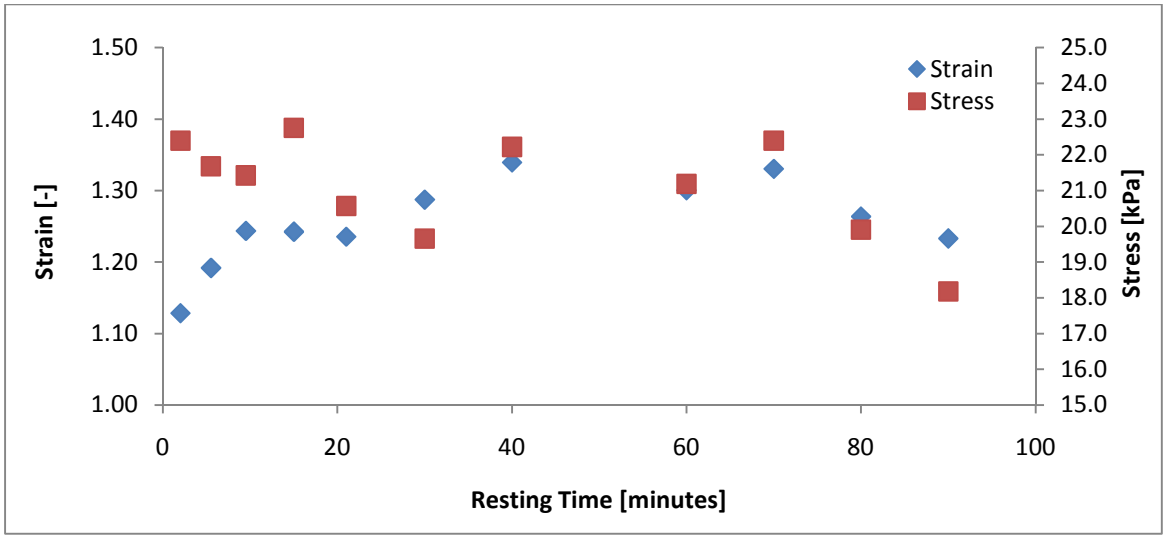


Figure 5.16. Stress and strain at sample failure for samples processed using standard conditions with different resting times.

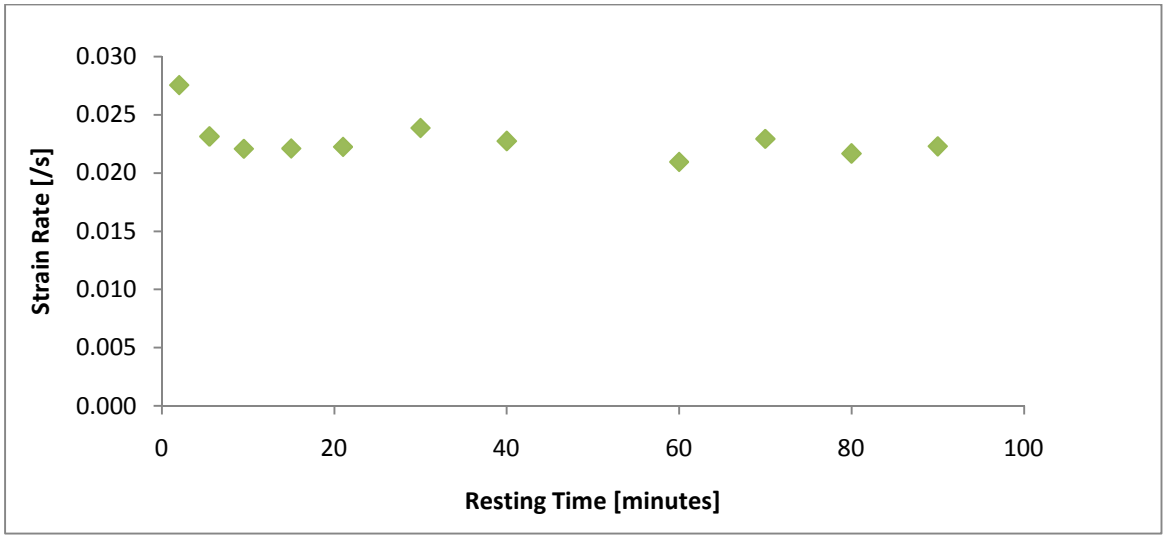


Figure 5.17. Strain rate at sample failure for samples processed using standard conditions with different resting times.

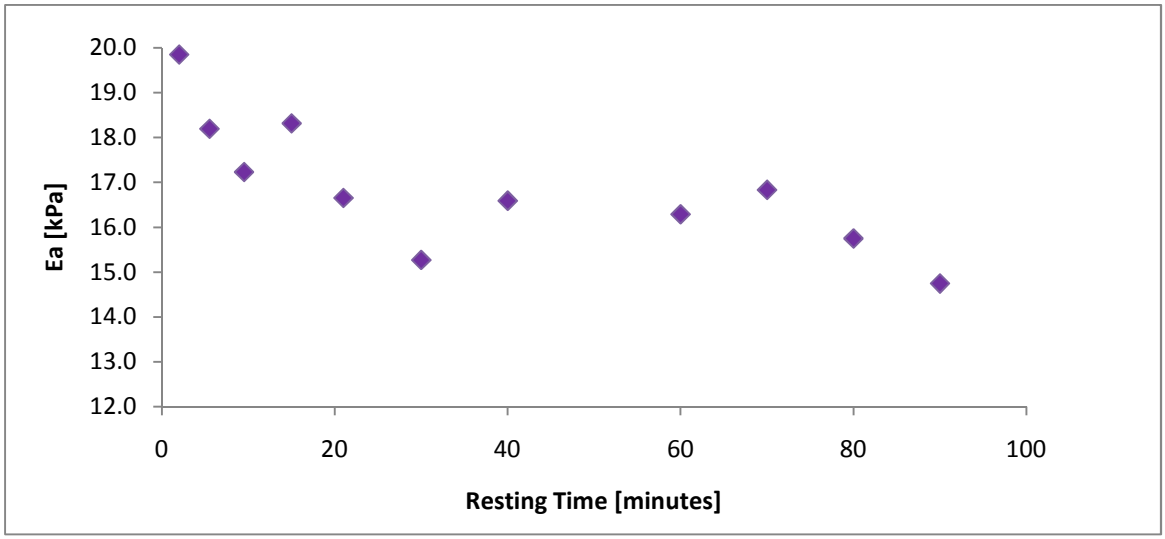


Figure 5.18. Apparent modulus of elasticity at sample failure for samples processed using standard conditions with different resting times.

Table 5.7 summarises stress, strain, strain rate, and apparent modulus of elasticity at the sample failure point for samples processed using various composition and processing conditions.

Table 5.7: Rheological Parameters at Sample Failure for Various Composition and Processing Conditions

Sample	Parameter	Stress (σ)	Strain (e)	Strain rate ($\dot{e}$)	Apparent modulus of elasticity (E_a)
1	Standard	24.0	1.21	0.023	19.8
3	Short kneading	17.8	1.21	0.023	14.8
2	Long kneading	26.4	1.25	0.022	21.2
12	Very long kneading	11.8	1.00	0.031	11.8
4	Low salt (0.01 %)	16.5	1.07	0.029	15.4
5	No salt (0.0 %)	16.2	1.09	0.025	14.8
6	Double rolling	19.0	1.12	0.025	17.0
7	Low gluten	13.0	0.92	0.033	14.2
8	High gluten	26.4	1.37	0.020	19.2
11	Small particle flour	17.4	1.26	0.022	13.8
10	Large particle flour	14.2	1.14	0.027	12.5

5.4. Discussion

5.4.1. Resting Time

It is generally accepted in literature for wheat dough, that the stresses introduced by kneading, handling, and sheeting are reduced during the resting time (this is usually called “dough relaxation”) [78, 161, 176]. In these investigations using sheeted oat-gluten dough, changing the dough resting time generated a force versus elongation graph with similarly shaped curves for the different resting times. However, the magnitude of the force changed as the resting time of the sheeted oat-gluten dough changed (Figure 5.8). Generally, as the resting time increased, the maximum force decreased.

Similarly, changing the resting time produced stress versus strain graphs that were similar in shape (Figure 5.11). All samples produced a curve that was concave upwards indicating that strain was increasing more rapidly than stress. This indicates that strain hardening was occurring. The slope of the curve differed between samples, with shorter resting times generally displaying a steeper curve (higher stress for any given strain). These results indicate that the rheology of the dough changed with changing resting time. Stress decreased with increasing resting time, showing that stresses within the oat-gluten dough were reduced over time. This shows that relaxation of oat-gluten dough occurred, similar to wheat dough. This finding is consistent with previously published data for sheeted wheat dough where the rheology of the dough sheets changed over a resting period [161]. This supports the hypothesis that during the resting period, changes (either chemical or physical) occurred in the protein network of the sheeted oat-gluten dough.

The rheological parameters strain hardening, strength index, and apparent modulus of elasticity all decreased with increasing resting time (Figures 5.13 to 5.15). All of the samples displayed a decrease in strain hardening, strength index, and apparent modulus of elasticity that was not constant or linear with resting time. The rate of decrease slowed with increasing resting time. The characteristic resting time calculation showed that this decrease was over a similar time period for strength index and

apparent modulus of elasticity (τ = 14.7 minutes and τ = 13.4 minutes). This indicates that oat-gluten dough resting had an effective endpoint after which further "resting" was not necessary. A resting time end point is valuable information for manufacturing, as it will allow the resting time to be defined, optimising production times. The existence of the resting time endpoint is consistent with previously published literature for sheeted wheat dough [161]. These authors reported that changes in apparent elongational viscosity of wheat dough levelled off after approximately 50 minutes.

These results support the hypothesis that during the resting period, changes (either chemical or physical) occurred in the protein network of the oat-gluten dough. These changes are likely to be similar to those that take place in the protein network of wheat dough. However, it is obvious from the difference in characteristic resting times between oat-gluten dough and that reported for wheat dough, that the changes during resting time in oat-gluten dough are more rapid. Therefore it was concluded that the changes that occurred in the oat-gluten dough are similar, but not identical, to the changes that occur in wheat dough.

5.4.2. Composition and Processing

Varying the composition and processing of the oat-gluten dough resulted in force versus elongation graphs with curves that were similar to each other and the resting time samples. The magnitude of the force was found to differ between the different samples (Figure 5.9). Compared to the standard composition and processing conditions; reducing the salt content, reducing the gluten content, reducing the kneading time, double sheeting, and altering the particle size distribution of the flour (larger and smaller) all resulted in a lower force for any given elongation.

Varying the composition and processing of the oat-gluten dough produced stress versus strain graphs that were similar in shape with curves that were concave upwards indicating that strain hardening was occurring (Figure 5.12 a to f). Altering the particle size distribution of the flour (larger or smaller) resulted in a lower slope than for the standard composition and processing (Figure 5.12 c). It is possible that the large particle size is slowing the hydration of the protein contained in the large particles and influencing the formation of the protein network. The smaller particle is

likely to contain more damaged starch granules with higher solubility and would alter the rheology. Reducing as well as increasing the kneading time resulted in a decrease in stress and strain (Figure 5.12 f). This indicates that there is an optimal kneading time and flour particle size. This supports the hypothesis that variations in the composition and processing history of the oat-gluten dough result in changes to the protein network (chemical or physical).

These results are consistent with results from other authors which have shown that changing the composition of wheat dough alters the rheology [107, 177]. The study by Zheng et al. [178] showed that the stress for any given strain for sheeted wheat dough reduced when the work input during mixing was increased or decreased from an optimum value. Other authors have also shown that the mixing energy and time influences the formation of the gluten protein network and dough rheology [81, 82, 179, 180]. Reducing agents facilitate the cysteine linkages (disulphide bonding) between the amino acids in the protein molecules of wheat dough and can effect changes that occur during dough resting and the rate that these take place [181].

The rheological parameters strain hardening, strength index and apparent modulus of elasticity were investigated for the effect of varying the composition and processing for the situation where $e = 1$ (Table 5.6). Only the sample kneaded 25 % longer than the standard (150 seconds) produced higher values for strength index and apparent modulus of elasticity. Three samples produced higher values for strain hardening compared to the standard; 25 % longer kneading, 25 % higher gluten, and large particle specification oat flour (<700 μm). Samples that produced low values for strain hardening, strength index, and apparent modulus of elasticity were; short kneading (25 % less), low gluten (25 % less), reduced salt (50 %), no salt, and small particle specification oat flour (<250 μm). Large particle specification oat flour also produced low values for strength index and apparent modulus of elasticity. These results show that kneading time, gluten content, and salt content are key factors in oat-gluten dough rheology. The results suggest that there is an optimal value for these composition and processing variables and is consistent with other published literature for wheat dough. It is apparent that over-kneading occurred in the oat-gluten dough kneaded for 3 minutes (Sample 12) which was weak.

The results from this research study on oat-gluten dough are consistent with other published research on wheat dough performance in baking and bread making [64, 77, 156, 177, 183]. The studies on wheat dough report that both short kneading time or low gluten content typically produce weak wheat dough that performs poorly in baking and bread making. Conversely longer kneading time or higher gluten content typically produce strong wheat dough that performs well in baking and bread making. Over-kneading can produce a weak and sticky wheat dough. Published research on wheat dough has shown that dough mixing has an optimum time [81, 82, 178, 180]. Other researchers have shown that salt content influences the formation of the protein network in wheat dough [25, 107, 182].

5.4.3. Failure Point

During these trials, some samples with obvious flaws were tested to observe the robustness of the measurement technique. It was observed that any flaw in the sheeted oat-gluten dough sample became the failure point and influenced the result. The dough was found to rupture prematurely if there was a flaw such as a crease or large air bubble in the dough. The rupture was observed to begin at the flaw. This makes sense, as a flaw is likely to be a weak point in the dough where the stresses and strains will become concentrated. This demonstrates the care that must be taken when preparing sheeted oat-gluten dough samples to discard any flawed samples prior to measurement. Care was taken to select samples for these investigtions with no obvious flaws.

Resting time

At the point when the oat-gluten dough sheet failed there was no obvious trend in the stress and strain results as a function of resting time (Figure 5.16). Both strain rate and apparent modulus of elasticity at the failure point showed an initial decrease with strain rate levelling off to approximately 0.022 s^{-1} for samples that were rested for at least 10 minutes and apparent modulus of elasticity levelling off for samples rested at least 20 minutes (Figures 5.17 and 5.18).

Other authors have demonstrated that sheeted wheat dough typically produces a strong relationship between large deformation rheology and resting time [161]. These authors reported that longer resting times produced a decrease in stress, strain, or strain rate values at failure. However, this research study on sheeted oat-gluten dough has shown that only the strain rate produced a strong trend. This was unexpected. It was concluded that stress and strain at the failure point are not a suitable mechanism for assessing the effect of resting time on the sheeted oat-gluten dough.

Composition and Kneading

For the samples varying the composition and processing of the oat-gluten dough, the failure point data generated very similar results for stress, strain, strain rate, and apparent modulus of elasticity compared to the results produced when e = 1. The samples with low gluten content (25 % less) or short kneading time (25 % less) both failed at comparatively low stress, strain, and apparent modulus of elasticity (Table 5.7). Short kneading time sample and the low gluten content sample had the lowest strain rate at failure (0.031 s^{-1} and 0.033 s^{-1} respectively) compared to the standard conditions. These results show that sheeted oat-gluten dough that had been under-kneaded or contained lower levels of gluten produced weak dough. This supports the results for e = 1.

The samples with high gluten content (25 % greater) and long kneading time (25 % greater) had higher stress and strain at failure than the standard. However, the apparent modulus of elasticity was lower for the high gluten content than for the long kneading time and standard samples. These results show that sheeted oat-gluten dough that was optimally kneaded or contained higher levels of gluten produced a strong dough. This supports the results for e = 1. Extending the kneading time further (50 % longer) resulted in a lower stress, strain, strain rate, and apparent modulus of elasticity. This agrees with the results for e = 1, which showed that over-kneading produced weak dough and is consistent with work published by others for wheat dough which showed that dough mixing has an optimum duration [81, 82, 178, 180].

5.4.4. General Discussion

These large deformation rheology results indicated that a protein network was formed in the oat-gluten dough. The results support the hypothesis that during the resting period, changes (either chemical or physical) occurred in this protein network. The results also indicate that processing and composition of the oat-gluten dough influenced these changes in the protein network.

The results show that the changes in the protein network of the sheeted oat-gluten dough appear similar, but not identical to those that take place in the protein network of wheat dough. The protein network in wheat dough comprises the glutenin and prolamin protein fractions and is formed during kneading [2, 6, 70]. It is held together by both inter- and intra- molecular bonds (mainly by disulphide linkages) as well as secondary bonding forces such as hydrogen bonding, ionic bonding and other non-covalent bonds. During resting period (immediately following kneading) the bonds rearrange to minimise internal stresses in the dough (dough relaxation). New bonds then form resulting in the changes to the protein network [25].

Similar to wheat dough, the composition and processing history of the oat-gluten dough resulted in changes to the protein network which was measured using rheology. Kneading time, gluten content, and salt content were key factors in formation of the protein network. As discussed previously, the large deformation rheology results were consistent with, but not identical to, other published literature for wheat dough. This suggests that the wheat gluten proteins present in the oat-gluten dough were the source of the protein network and this is a key conclusion of this research. The investigations did not identify if the oat proteins were involved in the protein network formation. Interactions between the oat and gluten proteins are the topic of Chapter 7 of this thesis.

It is proposed that blending the wheat gluten with oat flour disrupted the normal wheat gluten behaviour, affecting the protein network. The mechanism for protein network formation and dough resting for oat-gluten dough is expected to differ from wheat dough for several reasons. The composition of oat flour is different to wheat flour (for example higher protein, beta-glucan, and lipid content). Some of these components in

the oat flour are likely to have disrupted the interactions between the gluten proteins such as the formation of disulphide linkages and secondary bonds and this caused the gluten protein to relax more rapidly.

5.5.Conclusions

A key conclusion of these large deformation rheology investigations was that a protein network formed in the oat-gluten dough during kneading. Further investigations into the formation of a protein network in the oat-gluten dough are described in later chapters of this thesis.

The hypothesis for these trials was confirmed. The large deformation rheology showed that changes occurred in the protein network of the sheeted oat-gluten dough during the resting period that follows kneading and rolling/sheeting. This research also showed that the processing and composition of the oat-gluten dough influenced the rheology of the sheeted oat-gluten dough. These trials did not identify whether the changes were a result of either chemical or physical changes in the oat-gluten dough. Interactions between the oat and gluten proteins are the topic of Chapter 7 of this thesis.

Stress, strain, strain hardening, strength index, and apparent modulus of elasticity of sheeted oat-gluten dough were found to change with resting time. This is consistent with other data discussed in this thesis which has found that changing processing conditions influences the protein network formation as well as the starch and protein separation in the Al-Hakkak Process (discussed in Chapters 6 and 9).

It was concluded that during resting, changes (either chemical or physical) occurred in the oat-gluten protein network of the sheeted oat-gluten dough. A characteristic resting time of approximately 14 minutes was calculated for strength index and apparent modulus of elasticity. It was concluded that oat-gluten dough resting had an endpoint after which further “resting” was not necessary. The existence of such a resting time end point is valuable information for manufacturing, as it will allow the resting time to defined, minimising production times. It was proposed that the

characteristic resting time could be used to provide a mechanism for quantitatively determining the optimal resting time.

These results show that kneading time, gluten content, and salt content are key factors in oat-gluten dough rheology. Short kneading time or low gluten content produced weak oat-gluten dough. Conversely longer kneading time or higher gluten content produced strong oat-gluten dough. Over-kneading resulted in a weak dough. It was concluded that there is an optimal value for these composition and processing variables.

From these results it was concluded that the changes in the protein network of sheeted oat-gluten dough that take place during dough resting are similar, but not identical to the changes that occur in sheeted wheat dough. It was concluded that the wheat gluten proteins present in the oat-gluten dough were the source of the protein network. However, it was proposed that components in the oat flour disrupted the interactions between the gluten proteins such as the formation of disulphide linkages and secondary bonds. The investigations did not identify if the oat proteins were involved in the formation of the protein network. Interactions between the oat and gluten protein molecules are investigated in Chapter 7 of this thesis.

6. Oat-Gluten Protein Structure

6.1.Introduction

This chapter discusses investigations carried out on the large scale structure of the protein network formed in oat-gluten dough using the Al-Hakkak Process. Parts of this work were discussed in two conference papers presented at the 8th World Congress in Chemical Engineering, Montreal (2009), and the Xth International Gluten Workshop, Cleremont-Ferrand (2009) [50, 53].

As discussed in Section 2.5, wheat grain and the dough produced from this raw material is a multiphase material containing biopolymers such as starch granules, insoluble proteins, insoluble carbohydrates, lipids, and water soluble biopolymers (for example: sugars, beta-glucans, and proteins) [72, 185-187]. Various authors have shown that during the kneading process the structure of wheat dough changes as the gluten proteins interact to form a protein network typically referred to as the "gluten protein matrix" [25, 72, 174, 176, 188]. It has been shown that during wet separation processes the degree of wheat dough development influences the separation of starch from the protein network, such as the Martin Process and the Batter Process (discussed in Section 2.9) [56, 81, 180]. It is generally accepted that optimal wheat dough development creates a cohesive, visco-elastic, and open protein network structure [58]. The starch granules are clustered into pockets within the protein network which are readily released during extraction. Only a few individual starch granules are entrained in the protein network. Under-development of wheat dough generates small protein agglomerates and do not separate well from the starch granules during extraction. This results in contamination of the starch with small protein particles. Over-development of wheat dough creates a fine, uniform protein structure with the starch distributed evenly throughout the protein network. Many individual starch granules are entrained in the protein network which makes separation difficult.

Similar to wheat dough, oat-gluten dough produced from oat flour enriched with gluten flour is a multiphase material containing biopolymer fractions similar to those in wheat dough, although in different ratios (discussed in Section 2.5). The added complexity

of oat-gluten dough is the presence of both oat biopolymers (including proteins) and wheat gluten proteins. Previous studies on the Al-Hakkak Process have suggested that oat-gluten dough forms a protein network, similar to wheat dough [51, 52]. Oat starch granules can be separated from this protein network using an aqueous process, similar to the Martin Process. The large deformation rheology trials discussed in Chapter 5 also indicated that a protein network formed in the oat-gluten dough. However, the formation and structure of the protein network was not investigated and the location of the starch granules in oat-gluten dough was not known.

The hypothesis for this research was that a cohesive protein network forms when oat-gluten dough is kneaded. Processing steps, such as kneading, extraction and separation of the starch and protein fractions influence the formation and structure of the protein network in the oat-gluten dough and the location of starch granules trapped within it.

The aim of this study was to explore the effect of kneading, extraction, and separation of the protein and starch fractions on the structure of any protein network formed in oat-gluten dough and the location of residual starch granules in that structure. This was achieved by using confocal scanning laser microscopy to observe the influence of extraction time and different dough kneading times on the formation of a protein network in the oat-gluten dough. The focus of the trials was to observe the structure of any protein network that formed in the oat-gluten dough and the location oat starch granules trapped within it. The desired outcome was to establish if there were differences in the structure and arrangement of the protein network and the oat starch granules that could be correlated to processing data.

6.1.1. Confocal Scanning Laser Microscopy

Confocal scanning laser microscopy is a relatively new tool that has been used to analyse the structure of wheat dough in recent years [58, 72, 137, 138, 189]. It is a valuable tool for researchers investigating the structure of biobased materials such as dough that contain multiple components such as starch and protein. Described simply, the technique involves scanning samples through a single optical section using a laser

beam that is accurately focused to a specific focal plane section. Multiple scans at different focal planes are undertaken and the resulting images are stacked to produce a single image. For thick samples, these images are typically very clear compared to conventional microscopy which can result in blurring of the images due to out-of-focus areas. Three-dimensional (3-D) images can be built up by stacking images of different focal planes from the confocal scanning laser microscope. The resulting 3-D images can then be visualised, processed and analysed providing a greater insight into the structure of the material.

Confocal scanning laser microscopy imaging can be undertaken so that the individual components are imaged separately [58, 72, 137, 138]. Fluorescent molecules can be selected for the component materials of interest and used to label individual components. These molecules fluoresce under illumination by light of a specific wavelength emitting a known colour. This technique allows the individual components to be labelled and identified in the images. For dough analysis, confocal scanning laser microscopy allows the protein and starch to be stained green and red respectively. The wavelength of the laser light can be controlled to differentiate the starch and protein components clearly.

Confocal scanning laser microscopy was selected for these trials, as it has advantages over other techniques microscopy imaging techniques. Confocal scanning laser microscopy allows individual dough samples to be measured directly with minimal preparation of the samples reducing the risk of possible sample modification [138, 189]. Other microscopy techniques require samples to be highly processed. For example scanning electron microscopy requires samples to be dried, thinly sliced and coated with a thin layer of a conductive material such as gold. This can result in substantial modification of the structure of samples, particularly samples that contain high levels of moisture such as dough.

Confocal scanning laser microscopy using fluorescent labelling of the individual components does require some modification of the samples during staining [138]. The staining process involves wetting the surface of the sample with an aqueous solution containing the fluorescent staining molecules. Water soluble components in the

sample may be dissolved by the aqueous staining solution and other components, such as proteins and starch granules may swell in the presence of water. In this study, samples of the oat-gluten protein product from the Al-Hakkak Process were taken immediately following the initial aqueous extraction stage. Hence, the water soluble components present in the oat-gluten dough had already been removed. The insoluble protein fraction and any starch granules (especially damaged starch granules) were already swollen. Therefore the risk of sample modification during staining such as swelling or solubilisation of components was minimal. However, there was a risk of washing loose starch granules off the surface during the staining process. This was taken into consideration when the images were analysed.

6.2. Methodology

The oat-gluten protein product samples in these trials were prepared using the Al-Hakkak Process at shown in Figure 6.1 [51, 52].

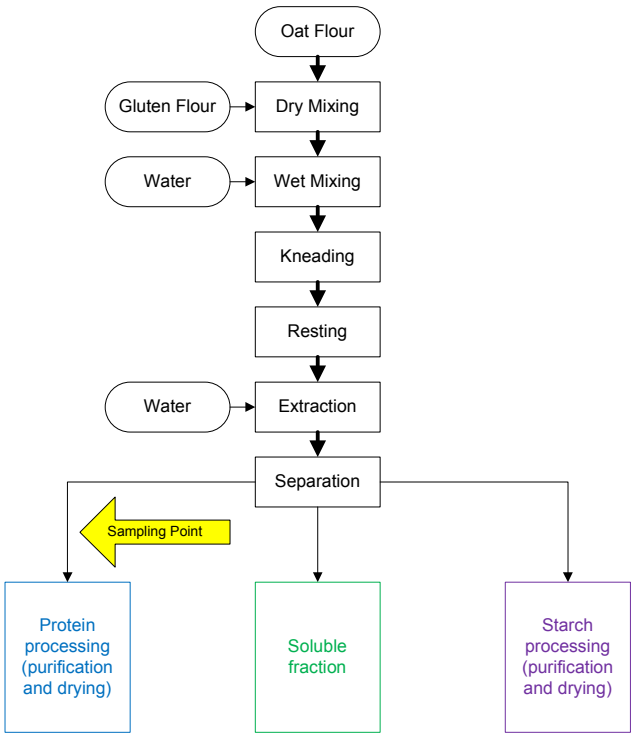


Figure 6.1. Schematic diagram of the Al-Hakkak Process showing the sampling point for the confocal scanning laser microscopy trials.

6.2.1. Equipment Selection

Samples were prepared using small pilot scale processing equipment that is similar in operation to large scale commercial processing equipment. Dry mixing, wet mixing and kneading were carried out using a Farinograph mixer fitted with a 50 g kneading vessel as described in Section 3.2. Extraction was carried out using 500 ml stirred, baffled vessels, with a pitched blade impellor as described in Section 3.2.

6.2.2. Sample Preparation

Four samples of the extracted oat-gluten protein were prepared for confocal scanning laser microscopy analysis. The samples were taken of the oat-gluten protein following the initial extraction (as shown in Figure 6.1), but before subsequent processing to purify the protein and remove the residual starch granules still trapped in the protein structure. This sampling location was selected as the initial extraction stage separates the most easily removable starch granules from the protein network. The starch granules are transferred to the extract liquor, forming a suspension (dilute slurry). Any starch granules remaining in the oat-gluten protein samples would be more tightly trapped within the protein network and would require further purification stages involving mixing in water to separate these. Thus, the protein network would be more easily observed and the location of the more tightly bound starch granules could be established. The influence of the extraction process could also be investigated.

Flour

Preparation and storage of the oat and gluten flour used in these trials is described in Section 3.3.

Dough Processing

Results from other trials in this study have shown that kneading time affects the purity of the oat-gluten protein from the Al-Hakkak Process (discussed in Chapter 9). Hence, the influence of kneading time on the protein structure and starch granule location was

investigated. Four oat-gluten dough samples were prepared using the Farinograph mixer following a standard recipe (Table 6.1). Three of the samples were produced using similar kneading conditions with only the kneading time varied between samples (Table 6.2). Samples 14 and 20 were kneaded for a standard duration of 120 seconds in the Farinograph mixer. Samples 15 and 16 had longer and shorter kneading times (150 seconds and 90 second respectively).

Table 6.1: Oat-Gluten Dough Recipe

Parameter	**Mass** (g)	**Dry basis** (%)	**Wet basis** (%)
Oat flour	48.10	80.2	47.8
Gluten flour	11.90	19.8	11.8
2 % Salt solution (NaCl)	2.0	-	2.0
Water	38.6	-	38.4
Water temperature (°C)	30	-	-

Table 6.2: Operating Conditions for Oat-Gluten Dough Preparation

Sample ID		**14**	**15**	**16**	**20**
Kneading temperature	°C	30	30	30	30
Wet kneading time	seconds	120	150	90	120
Resting temperature	°C	25	25	25	25
Resting time	minutes	90	90	90	90

Extraction

Similar to kneading, results from other trials in this study have shown that extraction time affects the purity of the oat-gluten protein from the Al-Hakkak Process (discussed in Chapter 9). Hence, the effect of extraction time on the protein structure was investigated. Table 6.3 describes the operating conditions used for the initial extraction of the oat-gluten dough to separate the oat starch granules from the insoluble oat-gluten protein network. The extraction time for Samples 14, 15 and 16 was 60 minutes. Sample 20 was processed using a reduced extraction time of 20 minutes.

Table 6.3: Operating Conditions for the Extraction Process

Sample ID		**14**	**15**	**16**	**20**
Dough mass washed	g	50.0	50.0	50.0	50.0
Water mass	g	200	200	200	200
Water temperature	°C	22	22	22	22
Extraction time	minutes	60	60	60	20

At the end of the extraction period, the extract liquor was poured over a 400 µm sieve and allowed to drain for three minutes. At the end of the draining period the oat-gluten protein on the sieve was carefully removed using a plastic scraper with minimal disturbance of the sample.

The oat-gluten protein from each sample was carefully placed into individual plastic containers and rapidly frozen by immediately immersing the sample in liquid nitrogen. The frozen samples were then transferred to a freezer for storage. Samples were flash-frozen to stop any further changes in the protein network taking place. Slow freezing would have allowed the protein samples to relax, potentially changing the structure of the protein network.

6.2.3. Oat-gluten Protein Structure Analysis

The structure of oat-gluten protein samples was analysed using an inverted Leica TCS SP5 confocal laser scanning microscope, located at the School of Biological Sciences, University of Canterbury.

The frozen samples of extracted oat-gluten protein product from the Al-Hakkak Process were carefully sliced into 1 mm thick slices using a razor blade to create a smooth surface for the confocal laser scanning microscope imaging. This removed the outer layer of each sample exposing the inner areas of the sample. This method removed any possible surface irregularities from the freezing process that would complicate interpretation of the results. Duplicate samples were placed on a cavity glass slide. The staining solutions were carefully placed onto the samples using a dropper. The samples were covered with a cover glass and stored in ambient

conditions for 30 minutes to allow the staining chemicals to react with the sample components.

A double staining technique was used to allow the simultaneous observation of the protein and starch components of each sample. This technique has been successfully used in other studies [58, 138, 189]. A combination of 0.1 g/L florescien isothiocyanate (FITC) and 0.1 g/L Rhodamine B in water was used to non-covalently label the starch and protein components respectively [72]. The excitation wavelength of FITC is 488 nm while Rhodamin B is 561 nm. In the resulting confocal scanning laser microscopy images the starch was observed as a green colour and the protein was observed as red. Empty spaces (voids) appeared black.

Images of the samples were observed with a 20x oil immersion objective. Three dimensional images of the protein fraction were generated by taking images of the sample over a depth of 20 µm at 1 µm intervals.

6.3. Results

6.3.1. Overall Appearance

Images were taken across each oat-gluten protein sample. These images were then stitched together to form a single large composite image of each sample, showing the overall appearance. Figures 6.2 to 6.5 show the composite images for Samples 14, 15, 16 and 20 respectively. Each image is a composite of eight individual images and the individual images that comprise the composite images can be found in Appendix C. Each composite image is 1560 µm by 780 µm. The images show that a protein network has formed in all of the samples and that the structure of this protein network differs between samples.

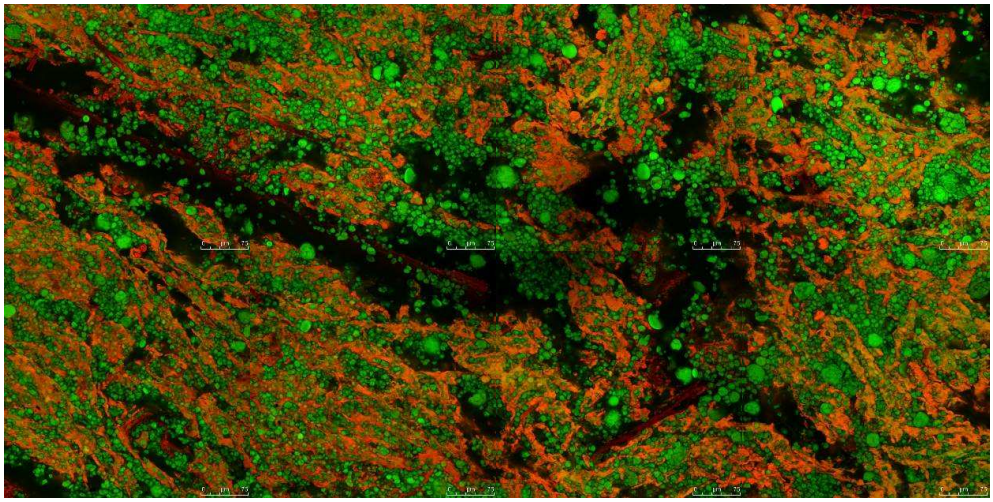


Figure 6.2. Overall appearance of Sample 14 (kneading time = 120 seconds).

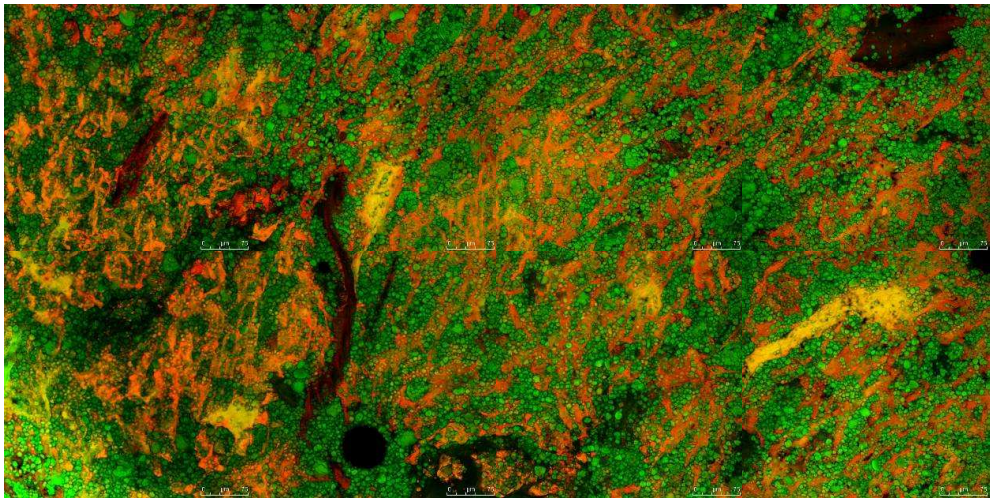


Figure 6.3. Overall appearance of Sample 15 (kneading time = 150 seconds).

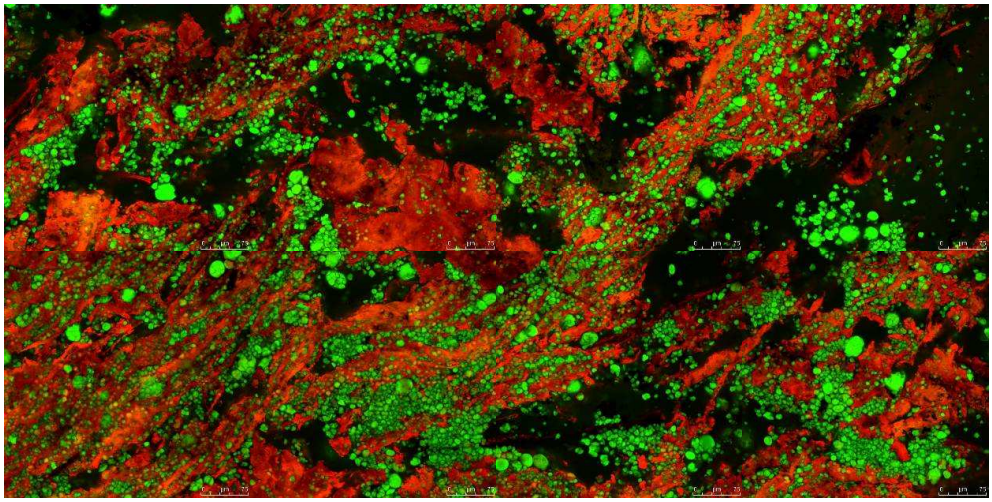


Figure 6.4. Overall appearance of Sample 16 (kneading time = 90 seconds).

Figure 6.5. Overall appearance of Sample 20 (extraction time = 20 minutes).

6.3.2. Protein Structure and Starch Location

High magnification images were used to observe the structure of the oat-gluten protein samples in detail, with particular focus on the structure of the protein network and the location of the starch granules. Figures 6.6 to 6.9 show detailed images for Samples 14, 15, 16 and 20 respectively, with the scale shown in the bottom right hand corner of each image. The images show that the structure of the protein network differs between

samples. The protein network in Sample 15 (longer kneading time) comprised fine, smooth protein agglomerates arranged into directional strands with individual starch granules imbedded in the protein agglomerates (Figure 6.7). The protein network in Sample 16 (shorter kneading time) consisted of large protein agglomerates and few elongated strands (Figure 6.8). The appearance of the oat-gluten protein network of Sample 14 (standard kneading time) was in-between Samples 15 and 16 (Figure 6.6). The protein network of Sample 20 (shorter extraction time) was loose and uneven with predominantly short, thick protein structures visible (Figure 6.9).

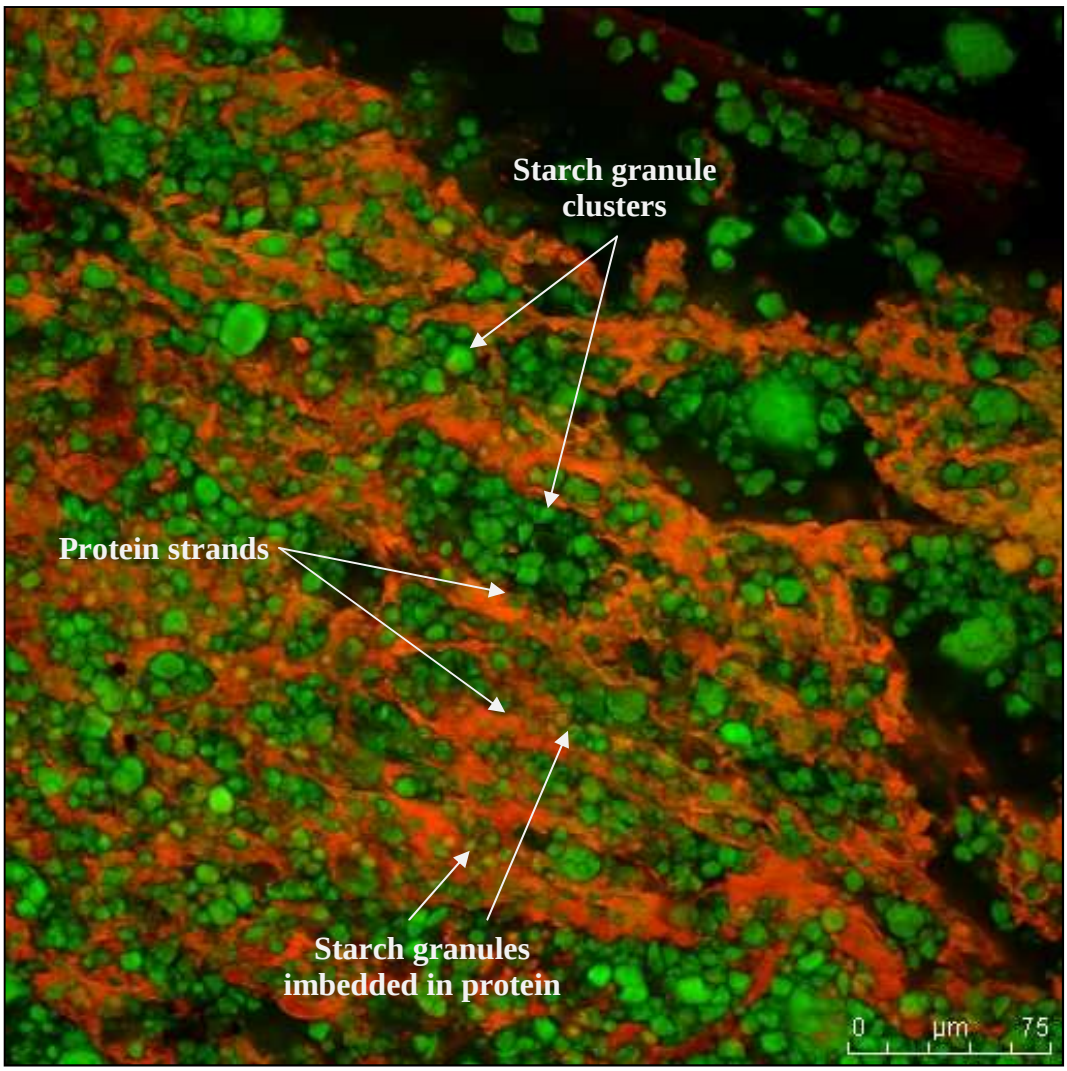


Figure 6.6. Detailed appearance of Sample 14 (kneading time = 120 seconds).

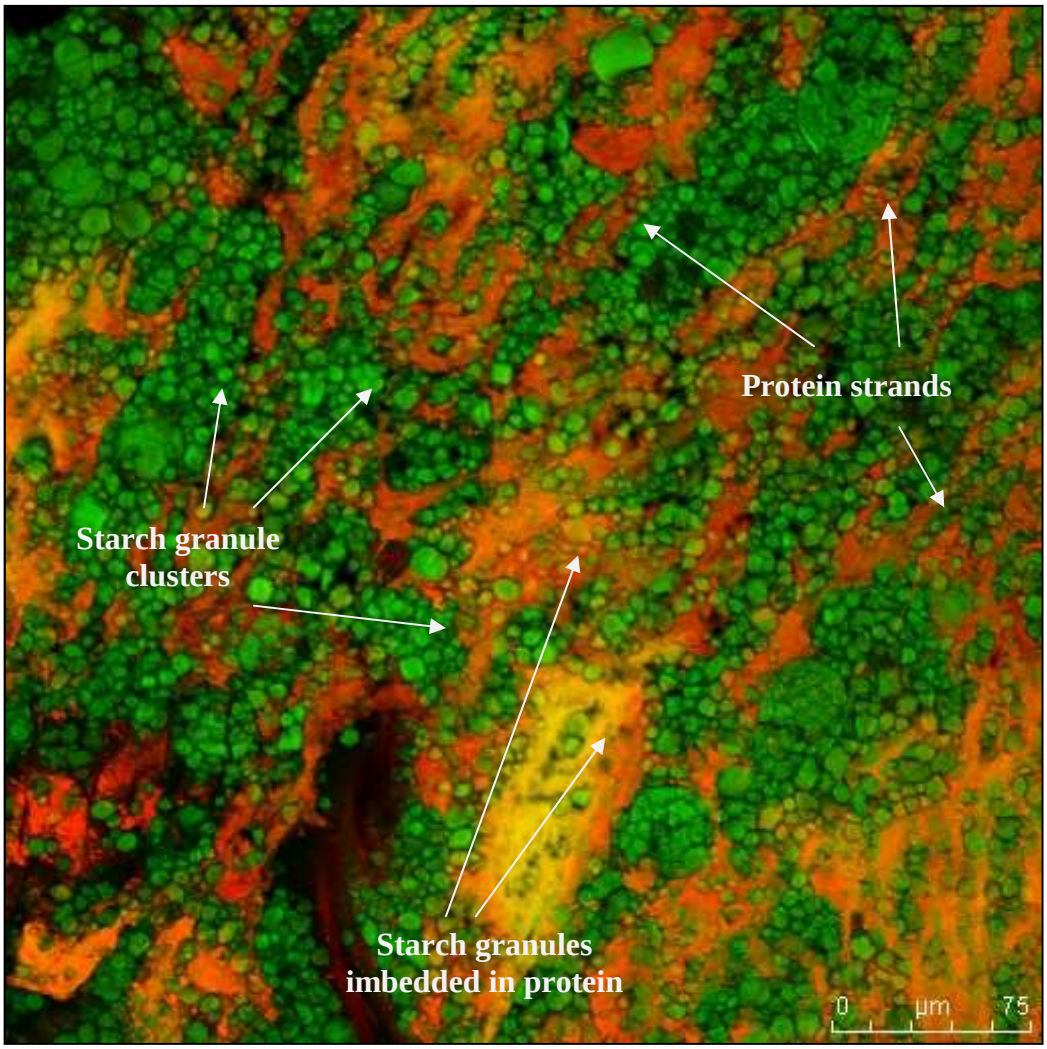


Figure 6.7. Detailed appearance of Sample 15 (kneading time = 150 seconds).

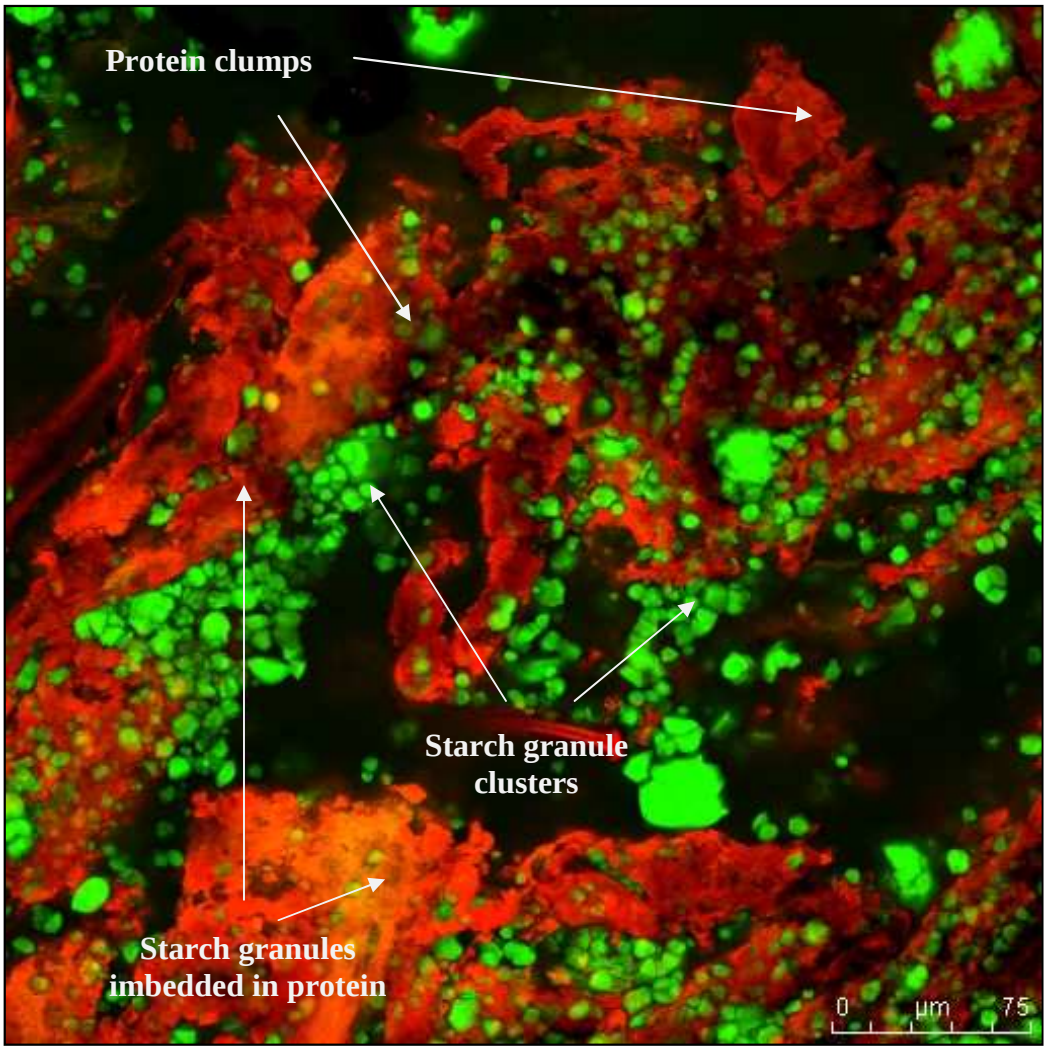


Figure 6.8. Detailed appearance of Sample 16 (kneading time = 90 seconds).

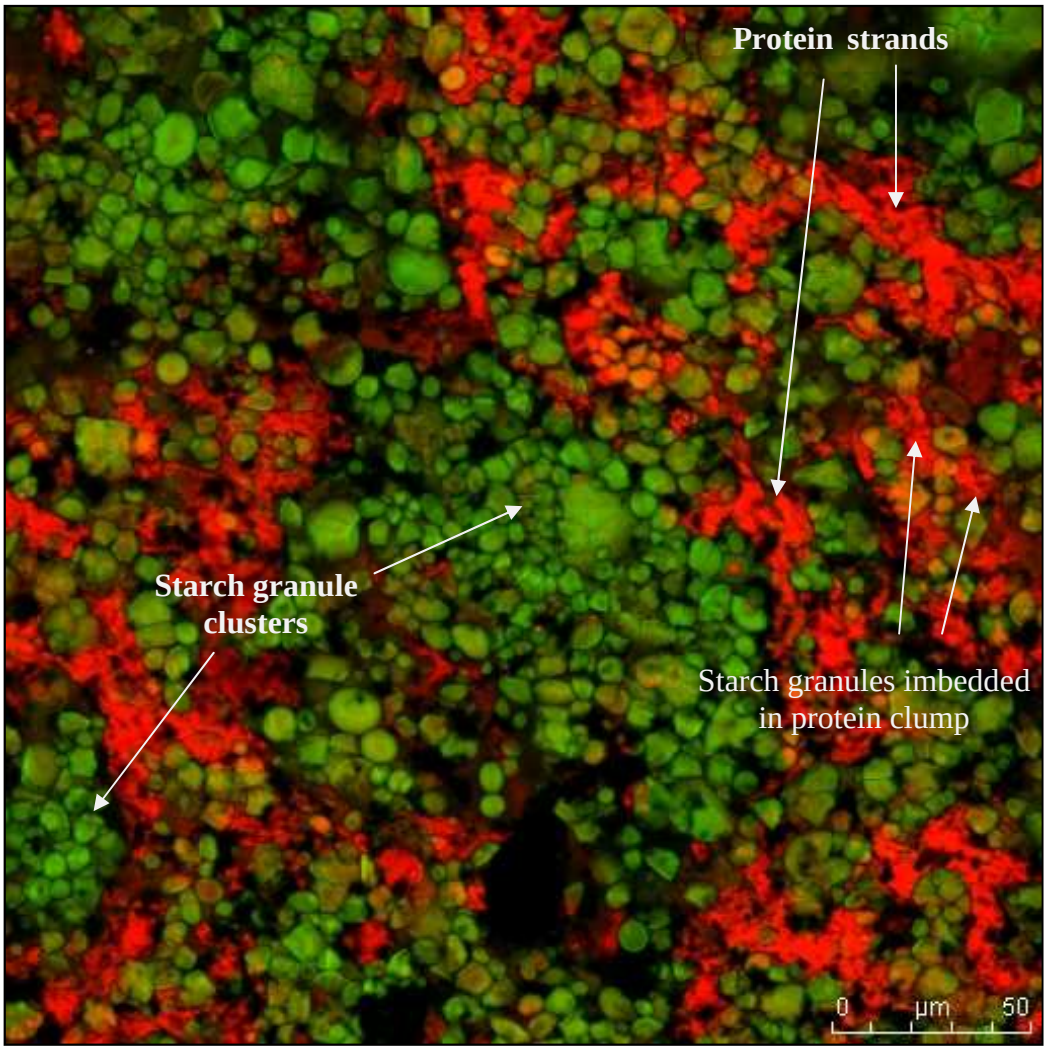


Figure 6.9. Detailed appearance of Sample 20 (extraction time = 20 minutes).

6.3.3. Protein Structure

Three dimensional (3-D) grey scale images of the protein component of each sample were generated to allow the structure of the protein network to be analysed and are shown in Figures 6.10 to 6.13. These images should be viewed using 3-D glasses.

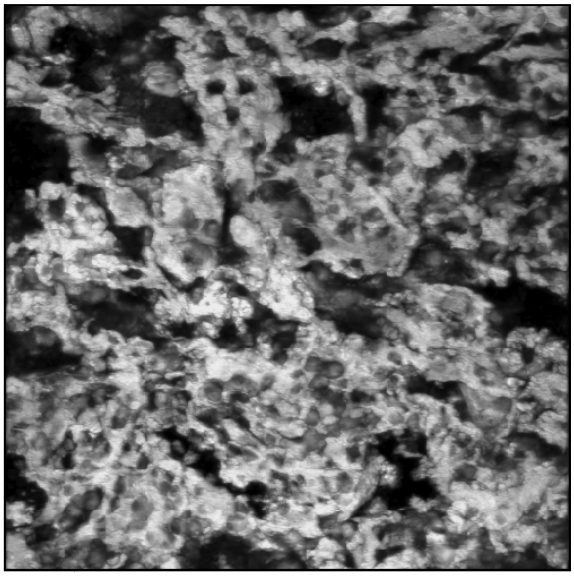


Figure 6.10. Protein structure of Sample 14 (kneading time = 120 seconds)..

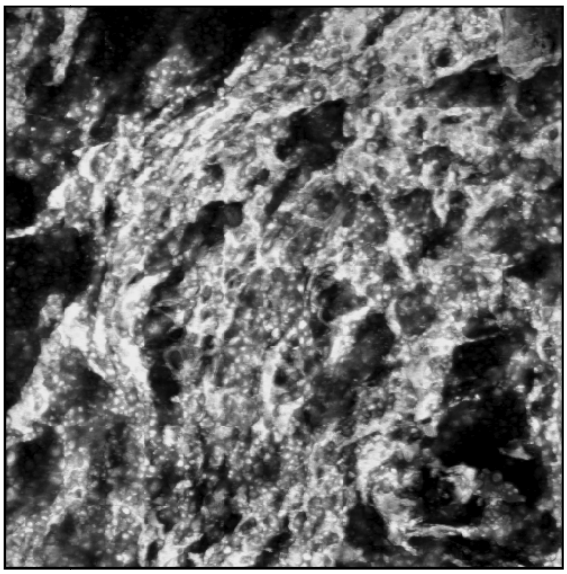


Figure 6.11. Protein structure of Sample 15 (kneading time = 150 seconds).

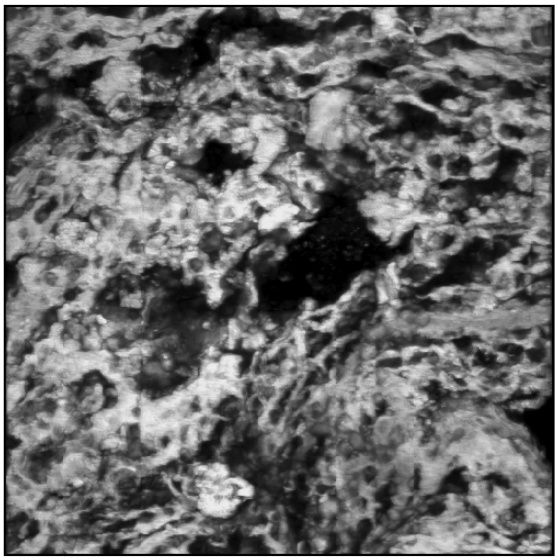


Figure 6.12. Protein structure of Sample 16 (kneading time = 90 seconds).

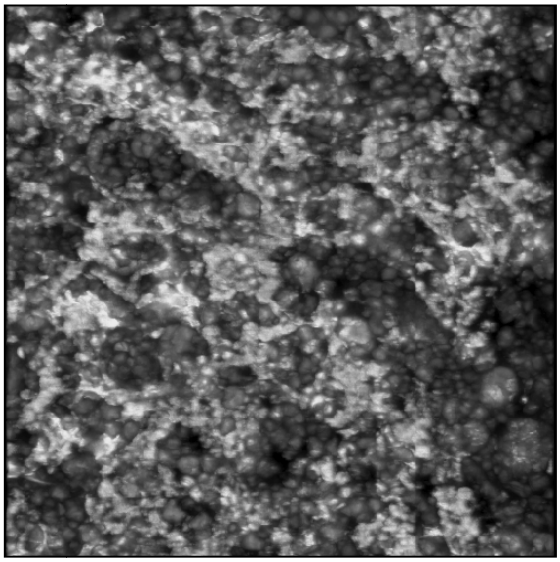


Figure 6.13. Protein structure of Sample 20 (extraction time = 20 minutes).

6.4. Discussion

Confocal scanning laser microscopy is generally considered a qualitative measurement tool. A large number of images are required to make a quantitative assessment that is robust and statistically significant. Also, as discussed in the introduction section of this chapter, loose starch granules may have been washed off the surface of the oat-

gluten protein samples during the staining process. Hence, the images of the oat-gluten protein samples from these trials were assessed qualitatively.

6.4.1. Protein Network Structure

The images from the confocal scanning laser microscopy confirmed the hypothesis that a protein network had formed in the oat-gluten protein samples. The structure of this protein network differed between the samples investigated. Differences were observed between the structure of the protein network of the four oat-gluten protein samples specifically the size and shape of the protein agglomerates that formed the protein network. Greater differences were observed when the extraction time was varied than the kneading time.

Kneading Time

The differences were observed in the protein network between Samples 14, 15 and 16 with different kneading times (120, 150, and 180 seconds kneading respectively). The protein network in Sample 15 (150 s) comprised fine, smooth protein agglomerates arranged into directional strands (Figures 6.3. and 6.7). This type of appearance has been associated with high development of the gluten protein network in wheat dough [137, 185]. The overall appearance of Sample 15 was relatively uniform, with protein and starch evenly distributed. The detailed image of Sample 15 showed individual starch granules imbedded in the protein agglomerates. The protein network in Sample 16 (90 s) had a rough and gritty appearance (Figures 6.4 and 6.8). The protein agglomerates were large and few elongated strands were visible. The overall protein structure of Sample 16 was uneven and loose, which is analogous of observations made in poorly developed wheat dough. Some individual starch granules imbedded in the protein agglomerates were observed in the detailed images of Sample 16, but less than Sample 15. The appearance of the oat-gluten protein network of Sample 14 (120 s) was in-between Samples 15 and 16 (Figures 6.2 and 6.6). Some smooth protein strands were visible, but these appeared less elongated than Sample 15. The protein strands in Samples 14 and 15 were generally well aligned, with strands running parallel to each other in areas of the samples.

It was concluded that the kneading time influenced the development of the protein network in oat-gluten dough. Hence, kneading time could affect the separation of starch granules from the oat-gluten protein network. Short kneading times would be expected to produce a coarse, poorly developed protein network that could collapse during the extraction process resulting in contamination of the starch with protein particles. However, long kneading times would be expected to result in a well developed and finer protein network, which entrapped individual starch granules making them difficult to separate. Hence, it was likely that there was optimum kneading time for the Al-Hakkak Process.

Extraction Time

Sample 20 (shorter extraction time) displayed the most visible difference in the protein network structure compared with the other three samples. The protein network of Sample 20 was loose and uneven with predominantly short, thick protein structures visible (Figures 6.5 and 6.9). This type of appearance has been associated with poor development of the gluten protein network in wheat dough [137, 185]. The protein network in the other three oat-gluten protein samples displayed elongated protein strings. This shows that the extraction time had an influence on the development of the protein network with a longer extraction time resulting in greater development. It was concluded that the extraction time influenced the development of the protein network in oat-gluten dough and that the effect of extraction time was more pronounced than kneading time.

A high degree of protein network development is undesirable, as it creates finer protein strands in the protein network and more evenly distributed starch granules. Individual starch granules become increasingly entrapped within the protein structure and more difficult to separate during the extraction process [185]. The confocal scanning laser microscopy images of the oat-gluten protein are consistent with this, showing a finer protein structure in the sample with the longest extraction time. It was concluded that the extraction step altered the structure of the protein network by increasing the protein development. This could affect the separation of starch granules from the protein network. Long extraction times would result in a well developed and finer protein

network, which would entrap individual starch granules. However, long extraction times would also provide more opportunity for the protein network to be opened up and release trapped starch granules. Hence, similar to kneading time, it was considered likely that there was an optimum extraction time for the Al-Hakkak Process.

It has been shown that for wheat dough that increasing the energy input during kneading increases the development of the gluten protein network in the dough [25, 71, 72]. This is consistent with the observations from this investigation on oat-gluten dough. During the extraction process, the oat-gluten dough was mixed with water in a stirred tank using a pitched blade impellor. More energy was imparted on the oat-gluten dough during this aqueous extraction and as a result there was additional development of the protein network. It was concluded that increasing the energy input by extending the kneading and/or extraction time increased the development of oat-gluten dough.

6.4.2. Residual Starch Granule Location

Pockets containing loose clusters of individual starch granules were evident in the protein network of all of the oat-gluten protein samples, although the size and shape of these pockets differed between samples. The starch granule pockets in Samples 14 and 15 (120 s and 150 s kneading respectively) where typically elongated (200 μm by 50 μm) and aligned with the protein strands (Figures 6.6 and 6.7). Sample 15 had considerably more individual starch granules entrapped within the protein agglomerates. Sample 16 showed fewer starch granule pockets which were generally larger in size (300 μm by 200 μm) (Figure 6.8). This indicates that kneading time influenced the location of the starch granules in the oat-gluten protein network.

This study has shown that in the early stages of kneading, the protein network formed short agglomerates, surrounded by large pockets of starch granules. As kneading progressed, the protein agglomerates formed into long strands and the starch pockets become smaller, with individual starch granules becoming increasingly embedded in the protein network. This suggests that there was an optimal kneading time for the Al-Hakkak Process. It is proposed that at this optimal kneading time the protein network

would have sufficiently formed to be cohesive and robust to be able to withstand the extraction process. Also at this optimal kneading time the starch granules would still be located in large and loose pockets and would not have dispersed into small pockets or individual granules entrapped within the protein agglomerates.

Considerably more starch granules were visible in Sample 20 (20 minutes extraction) compared to Sample 14 (60 minutes extraction) (Figures 6.9 and 6.6). Starch granules in Sample 20 were observed as individual granules entrapped in the protein network, clusters of granules located in pockets in the protein network, and as individual granules sitting on the surface of the sample. This indicates that more starch granules were separated from the protein network during the longer extraction (Sample 14). It was concluded that a minimum extraction time is required to separate the starch granules from the protein network in the Al-Hakkak Process. The minimum extraction time was not determined.

6.4.3. Protein Staining

Another observation in these confocal scanning laser microscopy investigations was that differences in the intensity of the red coloured stain of the protein network can be seen between the samples. The shorter kneaded Sample 16 (Figure 6.4) had a more intensely red stained protein network than the longer kneaded samples, which have a more yellow/orange appearance (Figures 6.2 and 6.3). All of the samples were stained using the same protocol and as such there should be no difference in colour. It is proposed that the longer kneading resulted in changes in the protein at a molecular level. Mixing is known to provide the energy to break bonds within the protein polymers that form the protein network of wheat dough, thus changing the molecular structure of wheat dough [64, 71, 74, 186]. Such a change in molecular structure could affect the functionality of the protein molecules and alter the propensity of the protein molecules to attach to the fluorescing molecules used in the staining technique. A change in molecular structure of the proteins could alter the uptake of the staining molecules by the proteins. This difference in the staining of the protein requires further investigation. Further investigation could include staining oat-gluten dough and wheat dough samples with different kneading times under carefully controlled

conditions (such as controlling staining temperature, time, and concentration). This could provide a useful colourimetric based method to evaluate dough development. This further investigation is beyond the scope of this study.

6.4.4. General Comment

The purpose of the confocal scanning laser microscopy was to establish if a protein network formed in the oat-gluten dough. A protein network was identified in the images. Differences were observed in the structure of the protein network when the kneading and extraction processes were varied. This confirmed the hypothesis that both kneading and extraction processes contribute to the formation of in the oat-gluten dough. However, the confocal scanning laser microscopy only provided a qualitative measurement of the oat-gluten protein network and further investigations using this technique were not considered worthwhile.

6.5. Conclusions

The confocal scanning laser microscopy investigations confirmed the hypothesis that a protein network had formed in the oat-gluten protein samples. This supports the outcome from the rheology investigations (Chapter 5) that showed that oat-gluten dough displayed visco-elastic properties that were similar to the properties of wheat dough and it was concluded that this was due to the formation of a protein network. However, the images did not distinguish between the oat and gluten proteins and it was not possible to establish if the oat proteins are involved in the formation of the protein network. Investigations into the interactions of the oat and gluten proteins are discussed in Chapter 7 of this thesis.

Analysis of the images from the confocal scanning laser microscopy provided valuable information on the influence of both kneading time and extraction time on the separation of starch granules from oat-gluten protein using the Al-Hakkak Process. Visually assessing the confocal scanning laser microscope images identified differences in the structure of the oat-gluten protein network and location of the starch

granules within the oat-gluten protein network as a result of changing kneading time and extraction time. A longer extraction time and/or kneading resulted in greater development of the oat-gluten protein network resulting in long protein strands and smaller pockets of starch granules within the oat-gluten protein network. Reducing the extraction time and/or kneading time increased the amount of starch granules present, indicating that the mixing action opens up the protein network and releases trapped starch granules. Two key conclusions were that there was an optimal extraction time and an optimal kneading time for the development of the protein network in oat-gluten dough.

Differences in the intensity of the protein staining were observed. It was suggested that mixing altered the protein molecules and as a result the ability of these molecules to attach to fluorescing molecules during staining changed. This observation requires further study.

This research has confirmed the hypothesis that both kneading and extraction contribute to the formation of the protein network in the oat-gluten dough. Both processes were found to influence the structure of the oat-gluten protein network and the location of starch granules trapped within that oat-gluten protein network. This research has shown that the effect of kneading time was not as pronounced as the effect of extraction time. A longer kneading time aligned the protein network into stringy, directional and smooth structure, whereas a shorter kneading time produced a protein network that was gritty with fewer directional strings. This is valuable information for the development and optimisation of a manufacturing process.

7. Oat-Gluten Protein Interactions

7.1.Introduction

This chapter discusses investigations carried out on the molecular interactions between the proteins that form the protein network of oat-gluten dough from the Al-Hakkak Process [51, 52].

As discussed in Sections 2.5.5 and 2.9.1 of this thesis, there are numerous studies of the molecular structure of wheat proteins, including wheat gluten proteins, and the chemical changes these proteins undergo during dough making. However, despite considerable research it is generally acknowledged that these changes are not well characterised and remain poorly understood [34, 180]. There is less information published on the molecular structure of oat proteins. The Osbourne solubility classes, typical molecular mass and typical amino acid composition are reported for oat proteins, following the methodologies established for wheat protein characterisation.

It was considered important to establish what role, if any, the insoluble oat proteins have in the formation of the protein network in the oat-gluten dough during the Al-Hakkak Process. No studies had been published on the molecular structure of oat proteins in dough or changes that oat proteins may undergo when hydrated and kneaded into a dough. Prior to research into the Al-Hakkak Process, no research had been published on combining oat and wheat protein, specifically adding wheat gluten flour to oat flour. There have been no studies on the molecular structure of proteins in oat dough enriched with wheat gluten proteins. A literature review focused on the molecular composition and structure of both wheat gluten and oat proteins has been included in this chapter which is more comprehensive than the general review included in Section 2.5.5 of this thesis.

The hypothesis for these trials is that physical entanglement occurs between the insoluble oat proteins and the wheat gluten proteins during the formation of the protein network. Thus, there is no chemical reaction (such as covalent, ionic, hydrogen, or other bonding) between the oat and gluten proteins in the protein network. Chemical

reaction between the wheat gluten and oat proteins is possible, due to the existence of possible reaction sites on both the wheat gluten and oat protein molecules (such as cysteine residues for disulphide bond formation). However, this is considered unlikely as it is thought that the molecular conformation of the wheat gluten protein and oat protein molecules is unfavourable for a reaction to occur. It is also likely that the potential chemical bonding pathways are disrupted by components in the oat flour.

The aim of this work was to establish if any chemical reaction occurred between the wheat gluten and oat proteins. It was proposed that gel electrophoresis would provide evidence of any interactions between the wheat gluten and oat proteins. Any changes in the molecular mass of the protein network could be identified by comparing the molecular mass distribution of proteins from the protein network formed in oat-gluten dough, gluten dough, and oat dough. Changes in solubility due to polymerisation or de-polymerisation could be identified by analysing both the insoluble protein network and the supernatant containing the soluble proteins. Analysis of the proteins from oat flour and gluten flour prior to processing would provide information on the protein structure prior to processing. The desired outcome was to demonstrate any changes in the molecular mass distribution of the insoluble protein in the protein network formed during the Al-Hakkak Process by comparing the oat-gluten protein network with both wheat gluten and oat protein.

7.1.1. Gel Electrophoresis

Gel electrophoresis has been widely used for the characterisation of the molecular mass of cereal proteins, including wheat proteins and oat proteins [40, 190-195]. This technique identifies the molecular mass distribution of the protein molecules due to differences in the electrophoretic mobility [34, 40, 192, 196, 197]. Protein polymers, including cereal proteins, often contain covalent bonds, such as the disulphide linkage that can be reduced yielding the basic protein subunits. Characterisation of the chemistry of the protein molecules is possible by applying gel electrophoresis techniques using both reducing and non-reducing conditions. Comparison between reducing and non-reducing conditions would provide the evidence of the presence of reducible covalent bonds in the protein polymers.

7.1.2. Protein Network Formation

It is well established that during the kneading process the structure of wheat dough changes as the gluten proteins interact to form a protein network typically referred to as the gluten protein network [25, 72, 78, 174, 176]. The formation of this visco-elastic protein network is unique to wheat dough. The characteristics of the proteins and the stages of the development of the gluten protein network are discussed in Section 2.9.1 of this thesis. However, the rheology investigations undertaken in this research project (discussed in Chapter 5) have demonstrated that oat-gluten dough has visco-elastic properties. The confocal laser scanning microscopy images in Chapter 6 have shown that a protein network formed in oat-gluten dough.

It is generally agreed in literature that various covalent and non-covalent bonds contribute to the formation of the gluten protein network in wheat dough [6, 34, 70, 73, 176, 192]. This network is formed by the interaction of the glutenin protein and gliadin protein fractions. The glutenin proteins are polymers comprising of high and low molecular mass sub-units linked primarily by disulphide bonds. The glutenin proteins have been shown to be responsible for the elastic rheological properties of the gluten protein network. The gliadin proteins are monomeric proteins of lower molecular mass and have been shown to provide the viscous rheological properties. The glutenin proteins have both inter- and intra-molecular disulphide bonds, whereas the gliadin proteins have only intra-molecular disulphide bonds.

During mixing in the presence of water, covalent and non-covalent bonds form, break, and reform between the gluten protein molecules in the wheat dough to form the protein network [34, 198]. This process is generally called dough development. Disulphide bonding is considered to be the dominant factor in the formation of the protein network and various models have proposed to describe how this process progresses during dough development through thiolate, mixed sulphide, and disulphide pathways [34, 74, 196, 199]. However, other bonds such as hydrogen, ionic, and hydrophobic bonds have been shown to influence the protein network formation [34, 104, 107, 200]. It has been proposed that for wheat dough, the disulphide bond stabilises the protein molecules, facilitating the formation of other bonds [198].

7.1.3. Wheat Protein Enrichment of Flour

Gluten enrichment of wheat flour, particularly for improving the bread and pasta making performance by enhancing the formation of the gluten protein network, has been widely reported in literature [25, 177, 191, 201-203]. This is a key use of "vital gluten", the gluten fraction isolated from wheat flour that has retained a high level of protein network-forming functionality. Gluten enrichment alters the rheology and improves the performance of bread, cookie, and pasta doughs. Wheat gluten is produced as a co-product of starch extraction using the various separation processes (discussed in Section 2.9 of this thesis).

The incorporation of other proteins into wheat flour (such as soy protein) has also been shown to alter the formation of the gluten protein network and the rheology of the resulting dough. However, in this case the dilution of the wheat gluten proteins with other proteins has been reported to negatively affected the performance of bread, cookie and pasta doughs [176, 198, 204]. Perez et al. [176] found that soy proteins had an adverse effect on the formation of the protein network during dough making. The overall effect was a weakening of the dough and the authors concluded that this was due to both physical and bonding interactions between the gluten and soy proteins. Marorimbo et al. [198] also found that the gluten and soy proteins interact through physical and bonding interactions, damaging the rheological properties of the dough.

Few studies have been reported on the incorporation of wheat gluten into flour from other cereals, seeds and legumes (such as rice flour). A recent study by Oszvald et al. [73] showed that the incorporation of wheat gluten into rice flour had a large effect on the mixing properties of the dough. However, the authors did not discuss the reasons for this.

7.1.4. Cereal Protein Molecular Mass

The molecular mass distribution of the various protein fractions is discussed (including references) in Section 2.5.5 of this thesis and a brief summary follows. Wheat gluten protein is comprised of glutenin proteins and gliadin proteins [34]. The wheat glutenin proteins are polymeric consisting of high molecular mass subunits of 80 to 130 kDa

and low molecular mass units of 10 to 70 kDa. The wheat gliadin proteins are monomeric with molecular masses of 35 to 70 kDa. Oat proteins vary in molecular weight from 5 to 70 kDa [40]. Oat avenin proteins (similar to gliadins in wheat) have a molecular mass range of 22 to 33 kDa. Oat glutenin are polymeric with large subunits of 32 to 40 kDa and small subunits of 20 to 25 kDa. Oat glutenin have been shown to have an almost identical molecular mass distribution as oat globulin and it has been suggested that these should be grouped together under a single glutenin classification.

7.2.Methodology

In these trials dough samples with different initial compositions were prepared following the Al-Hakkak Process as shown in Figure 7.1 [51, 52]. For each dough composition, samples of protein solids that comprise the protein network were taken after protein purification, but before drying. Corresponding samples of the soluble fraction were also taken.

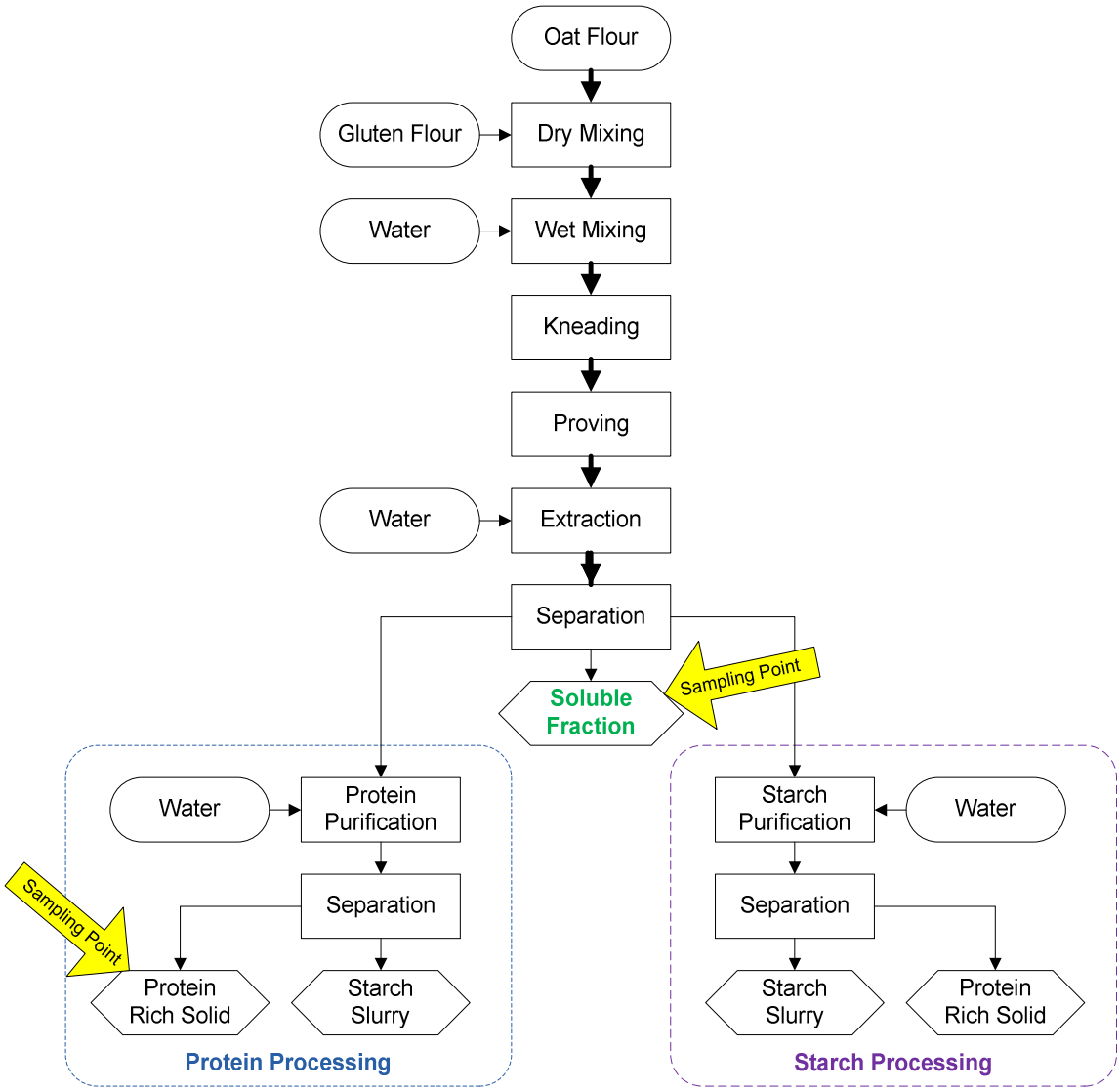


Figure 7.1. Schematic diagram showing the sampling point in the Al-Hakkak Process for these trials.

7.2.1. Equipment Selection

Samples were prepared using small pilot scale processing equipment that is similar in operation to large scale commercial processing equipment. Dry mixing, wet mixing and kneading were carried out using a Farinograph mixer fitted with a 50 g kneading vessel as described in Section 3.2. Extraction was carried out using 500 ml stirred, baffled vessels, with a pitched blade impellor as described in Section 3.2.

7.2.2. Sample Preparation

Three samples of the protein rich solids were prepared using the Al-Hakkak Process, from three different base flours oat-gluten flour, gluten flour, and oat flour.

Flour

Preparation and storage of the oat and gluten flour and wheat starch used in these trials is described in Section 3.3. Samples of the oat flour and gluten flour were taken for protein content testing and gel electrophoresis analysis.

Dough Processing

The three dough samples were prepared using the Farinograph mixer. The recipes for the three dough samples are shown in Table 7.1. The oat-gluten dough was prepared using a standard recipe combining both oat and gluten flour. The oat dough was prepared using the same recipe, but with the gluten flour omitted and replaced with additional oat flour. The gluten dough was prepared using only gluten flour, with the oat flour omitted and replaced with wheat starch. All other recipe conditions were held constant.

Table 7.1: Dough Recipes

Parameter	**Oat-gluten** (g)	**Oat** (g)	**Gluten** (g)
Oat flour	48.10	60.00	0.00
Wheat starch	0.00	0.00	48.10
Gluten flour	11.90	0.00	11.90
2 % sodium chloride solution (NaCl)	2.0	2.0	2.0
Water	38.6	38.6	38.6
Water temperature (°C)	30	30	30

All three samples were produced using the same kneading conditions (Table 7.2). After kneading the samples were put into individual, sealed, plastic bags, to minimise moisture loss during the resting period.

Table 7.2: Operating Conditions for Dough Preparation

Kneading temperature	°C	30
Wet kneading time [a, b]	seconds	120
Resting temperature	°C	25
Resting time	minutes	90

Extraction and Purification

All three samples were produced using the same extraction and purification protocols. Table 7.3 describes the operating conditions used for the initial extraction to separate the starch granules from the insoluble protein network.

Table 7.3: Operating Conditions for the Extraction Process

Dough mass washed	g	50.0
Water mass	g	200
Water temperature	°C	22
Extraction time	minutes	40

Water at 22 °C was placed into the extraction vessel and agitator positioned to the correct height (20 mm off the bottom of the vessel). The agitator was then turned on and the correct speed selected (corresponding to 120 rpm). A 50.0 g sample of dough was then cut into five pieces of similar size using hand scissors. These pieces were then dropped individually over a period of about 20 seconds into the agitated water.

A three stage purification process was used to remove the starch contamination from the insoluble protein network. This was the same for all three samples and Table 7.4 describes the operating conditions. For each sample the following protocol was applied. At the end of the extraction period, the extract liquor was poured over a 64 µm eve and allowed to drain for three minutes. The sieve was gently agitated during this draining period to minimise blinding of screen surface by the wet protein solids. At the end of the draining period the protein solids were carefully removed using a plastic scraper and returned to a clean extraction vessel. The total mass in this extraction vessel was made up to 250 g using 22 °C water. The agitator was then

positioned in the vessel with 20 mm clearance off the bottom and turned on. This process was repeated two more times.

Table 7.4: Operating Conditions for the Purification Process

Total mass washed	g	250.0
Water temperature	°C	22
Purification time	minutes	40

At the end of the third and final purification stage the protein solids from each dough sample were carefully removed from the sieve at the end of the draining period and placed into individual plastic bags which were placed in the freezer. At the end of the initial extraction the extract liquor was collected, covered and stored in a fridge at 4 °C for 4 hours. The starch granules settled forming a distinct white layer on the bottom of the storage vessel. The clear supernatant was carefully decanted off the top. This supernatant contained any soluble biopolymers extracted from the initial dough. An approximately 200 ml sample of the supernatant from each dough sample was frozen. The samples were frozen to minimise the risk of changes in the protein molecules during storage (such as microbial digestion).

7.2.3. Protein Molecular Interaction Analysis

The molecular interactions between the proteins were analysed using gel electrophoresis techniques for the various samples. The techniques used in this study involved sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). This method commonly used to separate proteins, including cereal proteins, according to their size [205]. The procedure for running an SDS-PAGE gel involves three main steps: 1) preparing the protein samples in the selected buffer containing SDS, 2) loading protein samples and running the gel, and 3) fixing and staining the separated proteins to make the proteins visible in the gel.

Both reducing and non reducing conditions were used in the preparation of the gels to identify the presence of any reducible covalent bonds such as the disulphide linkage. Initially the frozen samples of dough and supernatant were freeze dried. The flour

samples were not freeze dried as the moisture content was low. For the non-reducing conditions, 1 mg of protein was dissolved in 150 µl buffer (0.062 M tris, 10 % glycerol, 2 % SDS, 0.001 % bromophenol blue) and heated to 95 °C whilst being stirred. For the reducing conditions, 1 mg of protein was dissolved in 150 µl buffer (6 M urea, 0.062 M tris, 10 % glycerol, 6 % 2-Mercaptoethanol, 2 % SDS, 0.001 % bromophenol blue) and heated to 95 °C whilst being stirred. Reduced and non-reduced samples were run on separate 4 to 20 gradient SDS-PAGE gels purchased from a commercial suppllier. The gels were stained using Coomassie Brilliant Blue R-250 to make the protein bands visible. A set of commercially available marker molecules were used to provide a scale for the molecular mass of the protein bands and facilitate the interpretation of the gels.

7.2.4. Protein Identification

Mass spectrometry was used to identify the specific proteins that were present in selected protein bands from the non-reduced gels of the insoluble oat-gluten protein network and the gluten protein network. Protein bands of interest were identified and excised from the SDS-PAGE gel for analysis. Protein analysis was undertaken at the AgResearch Proteomics Analysis Facility using mass spectrometery to generate a fingerprint of the individual peptides present in the sample. This fingerprint mass spectrum was compared against a database of mass spectra for proteins and the proteins present in the original excised band were identified. This sample preparation, analysis and data interpretation was undertaken by an experienced mass spectrometry specialist operator.

7.2.5. Summary of Samples

Table 7.5 contains a matrix summarising the samples that were prepared for the molecular interaction investigations and describes the analysis that was carried out for each sample.

Table 7.5. Sample Summary

Sample	Analysis		
	SDS page reduced	**SDS page non reduced**	**Mass spectrometry**
Oat-gluten protein network	✓	✓	✓
Oat protein network	✓	✓	
Gluten protein network	✓	✓	✓
Oat-gluten soluble protein	✓	✓	
Oat soluble protein	✓	✓	
Gluten soluble protein	✓	✓	

7.3. Results

7.3.1. Gel Electrophoresis

The stained electrophoresis gels were examined and photographed. The disappearance, appearance or change in relative intensity of any of the protein bands was considered an indication of a change in the molecular mass of the proteins. Images were taken of the SDS-PAGE gels to provide a record of the molecular mass distribution of the different protein fractions from the Al-Hakkak Process. The protein molecular distributions of the oat-gluten, gluten, and oat protein network samples listed in Table 7.5 are shown in Figure 7.2 (a) (non-reduced conditions) and Figure 7.2 (b) (reduced conditions).

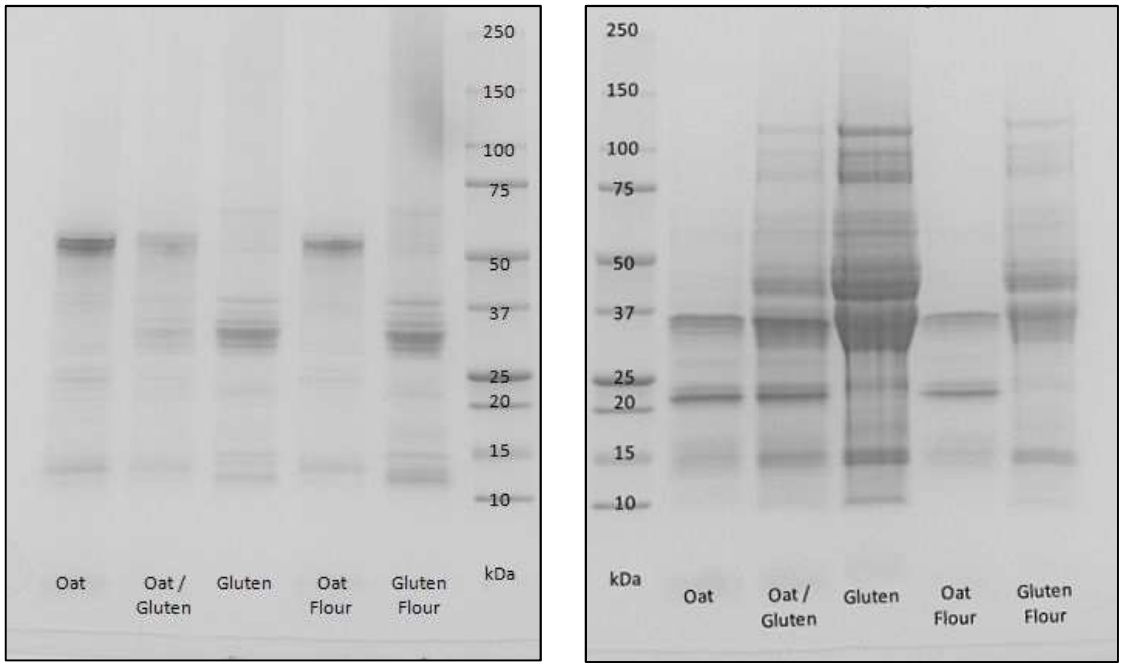


Figure 7.2: SDS-PAGE of the protein solids from the Al-Hakkak Process using oat-gluten dough, gluten dough, and oat dough as well as gluten flour protein and oat flour protein under (a) non-reduced conditions and (b) reduced conditions.

The reduced gels were initially compared for the oat flour protein and the oat protein network as well as the gluten flour protein and the gluten protein network. No differences in the location or relative intensity of the bands were observed. The protein band location and intensity observed in the non-reduced gel for extracted oat-gluten protein was compared with both the oat protein and gluten protein. Again, no differences in the location or relative intensity of the bands were observed.

The protein band location and intensity observed in the non-reduced gel for oat-gluten protein network was compared with both the oat protein network and gluten protein network and differences were observed between the samples. Specifically low molecular mass bands that were visible in the gluten protein network sample at ~12 kDa, ~14 kDa, and ~22 kDa and were absent in oat-gluten protein network sample. A high molecular mass band visible in the gluten protein network sample at 65 kDa was also absent in oat-gluten protein network sample. A protein band visible

in the oat protein network at ~55 kDa was relatively less intense in the oat-gluten protein network. These bands are difficult to see in the images of the gels. Figure 7.3 shows a schematic summary of the relative changes in the protein bands of the protein network from the Al-Hakkak Process in the non reduced gel.

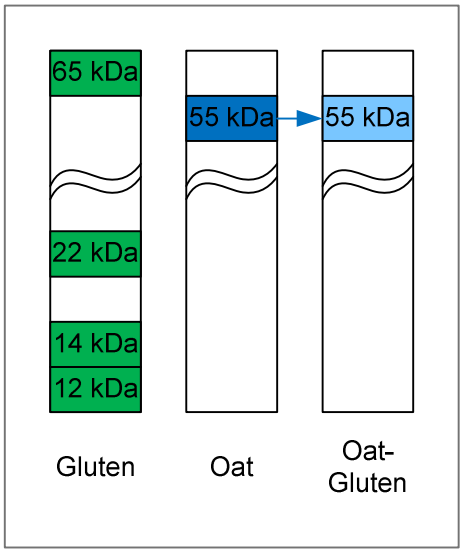


Figure 7.3: Schematic summary of the relative changes in the SDS-PAGE protein bands from the non reduced gel for oat-gluten, gluten, and oat protein networks from the Al-Hakkak Process (not to scale).

To investigate if proteins in the soluble fraction participated in the formation of the insoluble protein network, gels of the soluble protein from the Al-Hakkak Process were run under non-reduced conditions (Figure 7.4 (a)) and reduced conditions (Figure 7.4 (b))

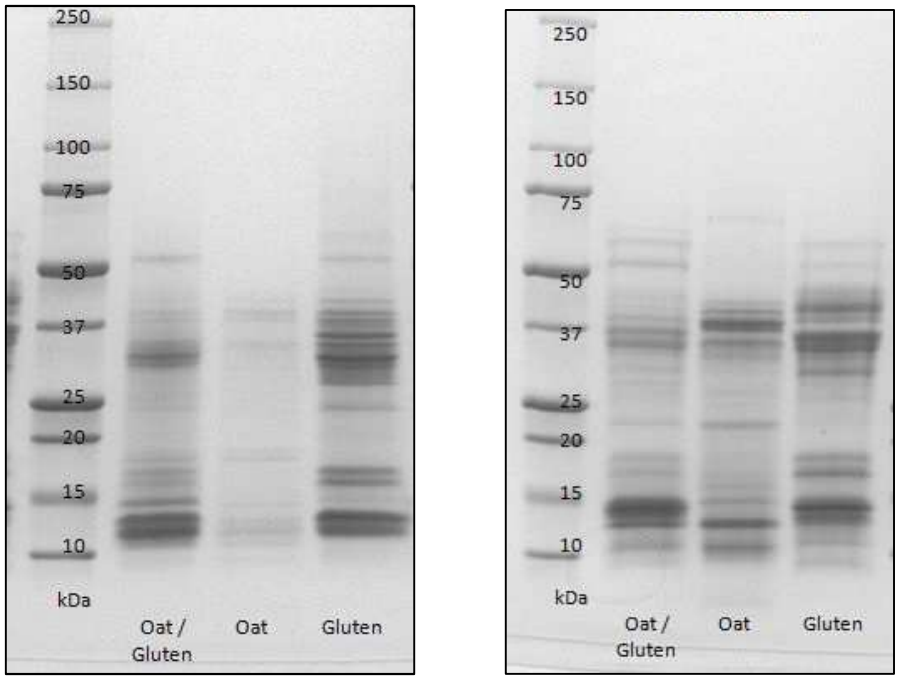


Figure 7.4: SDS-PAGE of the soluble proteins from supernatant produced by the Al-Hakkak Process, using oat-gluten dough, gluten dough, and oat dough under (a) non-reduced conditions and (b) reduced conditions.

The oat-gluten soluble protein was compared with both the oat soluble protein and gluten soluble protein in the reduced gels (Figure 7.4 (b)). Differences were observed between these soluble protein samples. Two protein bands that were visible in the oat soluble protein at ~16 kDa and ~36 kDa were absent in the oat-gluten soluble protein. A protein band present in the gluten soluble protein at ~42 kDa was also absent in the oat-gluten soluble protein.

The oat soluble proteins and the gluten soluble proteins were compared in non reduced gels and differences were observed between these samples (Figure 7.4 (a)). Two bands present in the gel for the gluten soluble protein at ~35 kDa and ~28 kDa were absent in the oat-gluten soluble protein. Two bands visible at ~37 kDa and ~42 kDa that were visible in the gluten soluble protein were relatively less intense in the oat-gluten protein. A low molecular mass protein at ~14 kDa has appeared in the oat-gluten soluble protein that is absent in both the oat soluble protein and gluten soluble protein.

These bands are difficult to see in the images of the gels. Figure 7.5 shows a schematic summary of the relative changes in the protein bands from the soluble proteins from the Al-Hakkak Process in the non reduced gel.

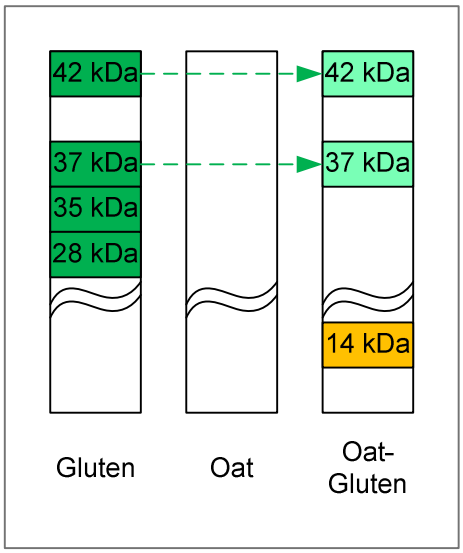


Figure 7.5: Schematic summary of the relative changes in the SDS-PAGE protein bands from the non reduced gel for soluble oat-gluten, gluten, and oat protein from the Al-Hakkak Process (not to scale).

7.3.2. Mass Spectrometry

Mass spectrometry identified two proteins in the bands excised from the gel for the oat-gluten protein network (dashed box in Figure 7.6). Two proteins were also identified for the bands excised for the gluten protein network (dotted box in Figure 7.6).

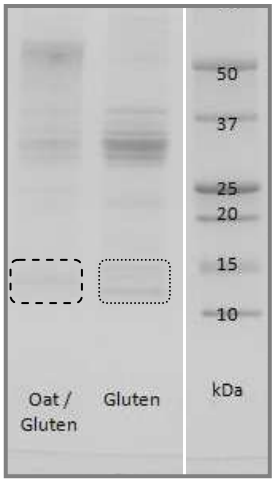


Figure 7.6. Excised bands from the SDS-PAGE of the oat-gluten and gluten insoluble protein solids from the Al-Hakkak Process, under non-reduced conditions.

The mass spectrometry results for the oat-gluten protein network are shown in Table 7.6 and the for gluten protein network in Table 7.7. The oat-gluten protein network contained both oat and gluten proteins (Table 7.6) and the gluten protein network contained gluten proteins from wheat (Table 7.7). Protein A from the oat-gluten protein network and Protein C from the gluten protein network are the same protein.

Table 7.6: Identification of Proteins for Oat-Gluten Protein Solids from the Al-Hakkak Process

Protein Band	**A**	**B**
Protein identification	Alpha-amylase/trypsib inhibitor CM3 precursor (Chloroform/methanol-soluble protein CM3)	Tryptophanin
pI	9.06	5.53
Theoretical molecular mass	18.21	16.88
Accession #	gi123957	gi155733229
Species	Triticum aestivum (wheat)	Avena sativa (oat)
No of unique peptides	6	3
Protein sequence coverage	35.71 %	19.05 %
Score	722.94	141.03

Table 7.7: Identification of Proteins for Gluten Protein Solids from the Al-Hakkak Process

Protein Band	**C**	**D**
Protein identification	Alpha-amylase/trypsib inhibitor CM3 precursor (Chloroform/methanol-soluble protein CM3)	Truncated puroindoline a
pI	9.06	9.22
Theoretical molecular mass	18.21	7.76
Accession #	gi123957	Gi149212449
Species	Triticum aestivum (wheat)	Triticum aestivum (wheat)
No of unique peptides	13	1
Protein sequence coverage	50.00 %	22.86 %
Score	791.83	216.55

7.4. Discussion

7.4.1. Gel Electrophoresis

Protein Network - Reduced Conditions

The protein band fingerprints produced for oat flour proteins, gluten flour proteins, gluten protein network and oat protein network (Figure 7.2 (b)) agree well with other previous published data [40, 76, 190, 192]. No differences were observed in the location or relative intensity of the protein bands of the various samples as a result of processing. This shows that there were no changes in the chemical structure of the oat proteins or the gluten proteins when processed separately using the Al-Hakkak Process.

To evaluate the protein bands observed in the reduced gel for oat-gluten protein network, the protein band locations and intensities were compared with both the oat and gluten protein network samples (Figure 7.2 (b)). No differences were observed. It was concluded that any molecular interactions between the oat and gluten proteins in the oat-gluten protein network must have involved reducible bonds. Thus, the reduced

oat-gluten proteins can be considered to be a simple combination of reduced oat proteins and reduced gluten proteins.

Protein Network - Non-Reduced Conditions

Gels were also run under non-reduced conditions to investigate the influence of the disulphide linkages between the proteins (Figure 7.2 (a)). It is widely agreed that various covalent and non-covalent bonds contribute to the formation of the gluten protein network but the disulphide bond is the primary contributor [34, 192, 196, 197]. The disulphide bond is preserved when non-reduced conditions are used. However, the solubility of the samples reduces. All of the protein network samples (oat, gluten and oat-gluten) prepared in these trials using non-reduced conditions showed reduced solubility. A proportion of the protein did not solubilise in the buffer during the preparation stage. For all of the non-reduced protein network samples (oat, gluten and oat-gluten) some large molecular mass protein remained at the top of each gel channel and was visible as a small dark line at the top of the gel (not shown). This was expected due to the large protein polymers held together by disulphide linkages remaining intact in non-reducing conditions. The glutenin protein polymer present in wheat gluten is reported to be large, approximately 3,000 kDa and held together by inter-molecular disulphide bonds [34, 43].

The non-reduced gels of the oat flour protein and the oat protein network as well as the gluten flour protein and the gluten protein network were compared. No differences in the location or relative intensity of the bands were observed for oat or gluten (Figure 7.2 (a)). This indicates that there were no changes in the chemical structure of the oat proteins or the gluten proteins when processed separately using the Al-Hakkak Process.

Differences were observed between the oat, gluten, and oat-gluten protein network samples in the non-reduced gel which suggested that new hybrid oat-gluten protein polymers were being formed. These differences are summarised in Figure 7.3 and discussed below.

- Low molecular mass bands that were visible in the gluten protein network at ~12 kDa, ~14 kDa and ~22 kDa and were absent in oat-gluten protein network. A high molecular mass band visible in the gluten protein network at 65 kDa was also absent in oat-gluten protein network. The absence of these protein fractions in the oat-gluten protein network suggests that the proteins interacted with other protein molecules to form new inter-molecular bonds. This resulted in a change in the molecular mass of the proteins molecules involved in the interaction. It is proposed that these new bonds formed between the gluten and oat proteins creating new hybrid oat-gluten protein polymers.
- Three bands visible at ~51 kDa, ~52 kDa and ~55 kDa were visible in the oat protein network, but the ~55 kDa band was relatively less intense than the others. This indicates that there were comparatively fewer ~55kDa proteins present. In oat-gluten protein network, all three bands were present with the same intensity. This relative increase in the intensity of the ~55 kDa band suggests that there was relatively more of the ~55 kDa protein fraction present in the oat-gluten protein network compared to oat protein network. It is likely that this was due to inter-molecular bonds forming between proteins creating ~55 kDa proteins or removing 51 kDa, and ~52 kDa proteins. Since this was only observed in the oat-gluten protein network it was proposed that new bonds have formed between the gluten and oat proteins creating new hybrid oat-gluten proteins.

Given that the reduced proteins were unchanged, it was concluded that the new bonds created in the oat-gluten protein network were reducible. Other studies have concluded that the glutenin fraction is comprised of two groups of polypeptides (α and β) which are linked by disulphide bonds in a hexamer arrangement [40]. The α and β oat glutenin polypeptides have different charge characteristics, with the β polypeptides being more basic. This confirms that disulphide bonding locations were available on oat protein molecules for linking to the gluten protein molecules during the Al-Hakkak Process.

Soluble Protein - Reduced Conditions

Similar to the insoluble protein network, the molecular mass distribution of protein samples from the soluble fraction from the Al-Hakkak Process was also investigated (Figure 7.4 (b)).

Differences were observed between these soluble protein samples in reduced conditions were observed. Two protein bands that were visible in the oat soluble protein sample at ~16 kDa and ~36 kDa were absent in the oat-gluten soluble protein sample. A protein band present in the gluten soluble protein at ~42 kDa was also absent in the oat-gluten soluble protein sample. The absence of these protein fractions in the oat-gluten soluble protein suggests that the proteins formed new bonds with other molecules, resulting in a change in the molecular mass distribution of the protein fractions. These bonds could have formed with other molecules in the soluble fraction (protein or carbohydrate) or with insoluble proteins. Running gel electrophoresis samples of the carbohydrate fraction of the soluble would indentify if the soluble carbohydrates were involved in the reaction. This was beyond the scope of this research project.

It was concluded that the changes in the molecular mass distribution of the soluble protein fraction (reduced conditions) was due to the formation of non-reducible bonds.

Soluble Protein - Non-reduced Conditions

None of the non-reduced soluble protein samples (oat, gluten and oat-gluten) displayed any evidence of large molecular mass proteins (a small dark line visible at the top of the gel). This shows that all of the soluble protein fractions are of lower molecular mass than the insoluble proteins.

Differences were observed between the soluble protein samples in the non-reduced gel, suggesting that the soluble proteins were involved in the formation of the new hybrid

oat-gluten protein network (Figure 7.4 (a)). These differences are summarised in Figure 7.5 and discussed below.

- Two low molecular mass bands present in the gel for the gluten soluble protein at ~35 kDa and ~28 kDa were absent in the oat-gluten soluble protein. This suggests that these protein fractions have interacted forming inter-molecular bonds with other protein molecules and creating new protein molecules. Since this was only observed in the oat-gluten soluble protein it is proposed that these new bonds have formed between the gluten and oat proteins creating new hybrid oat-gluten protein polymers.
- Two bands visible at ~37 kDa and ~42 kDa that were visible in the gluten soluble protein were relatively less intense in the oat-gluten soluble protein. These protein bands were absent in the oat soluble protein. This suggests that there were comparatively less of these proteins present in the oat-gluten soluble protein. It is likely that this was due to ~37 kDa and ~42 kDa protein molecules forming inter-molecular bonds with other protein molecules creating new proteins. Since this was only observed in the oat-gluten protein it is proposed that these new bonds have formed between the gluten and oat proteins creating new hybrid oat-gluten protein polymers.
- A low molecular mass protein at ~14 kDa appeared in the oat-gluten soluble protein that is absent in both the oat soluble protein and gluten soluble protein. This suggests that a new low molecular mass protein fraction has formed. This new protein fraction is only present in the non-reduced sample, so is the result of the formation of reducible bonds between protein subunits, such as the disulphide bond. The oat and gluten soluble protein samples both showed very low molecular mass bands (polypeptides). These were also visible in the oat-gluten soluble protein. It is likely that reducible bonds formed between some of these low molecular mass oat and gluten soluble polypeptides creating the new protein fraction.

No new large molecular mass protein bands were observed in the gel for the oat-gluten soluble protein and no relative increase in the intensity of any of the large molecular mass bands was observed. Hence it is concluded that the new bonds have formed

between the soluble proteins and the proteins in the insoluble protein network. This supports the conclusion that new reducible bonds were created between the oat and gluten proteins in the insoluble protein network creating new hybrid oat-gluten proteins. These results have provided evidence that the soluble proteins from the oat and gluten flour were involved in this interaction.

7.4.2. Mass Spectrometry

The mass spectrometry supported the conclusions from the gel electrophoresis, that both wheat and gluten proteins were present in the oat-gluten protein matrix. The mass spectrometry identified two proteins from the oat-gluten protein network as a protein from wheat (A) and a protein from oat (B) (Table 7.6) with molecular masses of 18.21 kDa and 16.88 kDa respectively. The protein from gluten protein network from the non-reduced gel contained two proteins (C and D), both from wheat (Table 7.7) with molecular masses of 18.21 kDa and 7.76 kDa. Protein A from the oat-gluten protein network and Protein C from the gluten protein network are the same protein.

7.4.3. Overall Discussion

Recent studies into the addition of soy proteins to wheat flour have shown that the wheat gluten and soy proteins interact [176, 198, 204]. Perez et al. [176] found that that after kneading, the soy proteins became associated with the insoluble wheat gluten proteins. The protein profile obtained using SDS-PAGE confirmed the presence of soy proteins in the extracted insoluble wheat gluten. These authors concluded that the association was through physical interaction, covalent bonds and other bonds occurring during dough kneading and resting. These interactions produced large and medium sized protein polymers and had a negative impact on the rheology of the wheat dough. Marorimbo et al. [198] also found that physical and bonding interactions occurred between soy proteins and wheat gluten proteins during dough making. Size exclusion HPLC showed that the soy globulins interacted with the insoluble wheat gluten proteins forming aggregates of high molecular mass. Capillary electrophoresis revealed that reducible bonds had formed, and the authors suggested that these were probably disulphide bonds.

This research into oat and gluten protein interactions has investigated the effect of the addition of wheat gluten to oat flour. The results are consistent with the previous studies reported by other authors discussing the incorporation of soy proteins into wheat flour. The gel electrophoresis results have shown that new reducible bonds, most likely disulphide bonds, occurred between the oat and gluten proteins that were present in the insoluble oat-gluten protein network during the Al-Hakkak Process. Changes were also observed in the soluble oat-gluten protein fractions.

7.5.Conclusions

The results from these trials disprove the hypothesis that only physical entanglement occurs between the insoluble oat proteins in the protein network formed by the wheat gluten proteins. The gel electrophoresis has shown that reducible chemical reactions occurred between the wheat gluten and oat proteins during the Al-Hakkak Process which involved both the insoluble and soluble protein fractions from the oat and gluten flour.

Differences were observed in the non-reduced gels for insoluble protein network and soluble protein fraction from the Al-Hakkak Process (identified by comparing oat-gluten protein with oat protein and gluten protein). Differences in protein fractions in reduced conditions must be due to the formation of non-reducible bonds such as the disulphide linkage. A new low molecular mass protein fraction was observed in the non-reduced, oat-gluten soluble protein. Proteins observed in the oat and gluten protein networks were absent or reduced in the oat-gluten protein network.

A key conclusion from this research was that during the Al-Hakkak Process the oat and gluten proteins interacted to form new reducible inter-molecular bonds, most likely disulphide linkages. A key discovery was that soluble proteins were involved in this interaction. It is proposed that these reducible bonds coupled oat and gluten protein subunits to form new hybrid oat-gluten protein molecules. The Al-Hakkak Process provided the necessary conditions (water and energy during mixing) for the protein coupling to occur. Further research is required to establish which specific

proteins and amino acid groups are involved in the protein coupling. This would identify the specific inter-molecular bonds that were forming and the structure of the new hybrid oat-gluten protein molecules. This investigation is beyond the scope of this research project.

8. Oat-Gluten Protein Functionality

8.1.Introduction

This chapter discusses investigations carried out the potential for reusing the insoluble oat-gluten protein network from the Al-Hakkak Process by substituting this for wheat gluten protein in subsequent Al-Hakkak Process batches. A key factor for the successful reuse of the oat-gluten protein is establishing the protein network-forming functionality of the oat-gluten protein compared with the original wheat gluten protein.

As discussed in Section 2.11 of this thesis, the Al-Hakkak Process requires the addition of wheat gluten protein to enable the separation of the oat starch granules, from the insoluble oat-gluten protein fraction as well as the soluble biopolymers present in the oat flour. Gluten flour is expensive and is currently about four times the cost of wheat flour and oat flour. Like all flour products, wheat gluten is considered a commodity product and the price fluctuates depending on geographical location as well as world market supply and demand. Recently wheat gluten has been selling at about ~NZ$4.10/kg [206]. This compares with wheat flour at about ~NZ$1.20 and oat flour at about ~NZ$1.35 from the same supplier. Substituting the insoluble oat-gluten protein product from the Al-Hakkak Process in place of wheat gluten could improve the economics of the commercial scale process by reducing the raw material costs. However, as shown in Chapter 7 of this thesis, the insoluble protein produced by the Al-Hakkak Process is not pure wheat gluten. It is a hybrid protein consisting of wheat gluten proteins and oat proteins. The effect of this oat protein component on the functionality of the wheat gluten was not known.

The hypothesis for this study was that some of the wheat gluten used in the Al-Hakkak Process can be replaced with insoluble oat-gluten protein isolated from a previous Al-Hakkak Process batch.

The aim of this study was to explore the impact of substituting wheat gluten with the insoluble oat-gluten protein from the Al-Hakkak Process. The focus for the investigations was comparing the functionality of the oat-gluten protein with that of the original wheat gluten protein. Specifically, the possibility for the oat proteins to

reduce the gluten functionality through dilution was explored. This was achieved by investigating the separation performance of the Al-Hakkak Process using recycled oat-gluten protein, by substituting various proportions of wheat gluten protein with insoluble oat-gluten protein from a previous Al-Hakkak Process batch.

8.1.1. Protein Network Formation

As discussed in Section 2.9.1, wheat gluten proteins interact and agglomerate to form a cohesive protein network during dough kneading [25, 72, 78, 174, 176]. The formation of this visco-elastic protein network is unique to wheat dough and does not occur in dough produced from other cereals such as oat. It is generally accepted that the development of the gluten protein network is due to the formation of various covalent and non-covalent bonds between individual wheat protein molecules [6, 34, 70, 73, 176, 192]. Specifically, this network is formed by the interaction of two wheat protein fractions (the glutenin protein fraction and gliadin protein fraction) primarily through disulphide bonding. The glutenin proteins (responsible for the elastic rheological properties of dough) are polymers comprising of high and low molecular mass sub-units which are linked primarily by disulphide bonds. The gliadin proteins are monomeric proteins of lower molecular mass (imparting the viscous rheological properties to dough).

In wheat dough, during mixing in the presence of water, covalent and non-covalent bonds form then break and reform between the gluten protein to form the protein network [34, 198]. Disulphide bonding is generally accepted to be the dominant factor in the formation of the protein network. Various models have been proposed to describe how this process progresses during dough development through thiolate, mixed sulphide and disulphide pathways [34, 74, 196, 199]. Other bonds such as hydrogen, ionic, and hydrophobic bonds have also been shown to influence the protein network formation [34, 104, 107, 200].

Similar insoluble protein fractions are present in oat flour but these do not interact during dough making and bonds do not form between the oat protein molecules.

However, research undertaken on the Al-Hakkak Process and presented in Chapters 5, 6, and 7 of this thesis suggest that the added wheat gluten proteins interact with the oat proteins to form a dough that is similar to wheat dough. This research has shown that oat-gluten dough has visco-elastic properties, similar to wheat dough (Chapter 5). Confocal scanning laser microscopy provided evidence that a protein network forms in the oat-gluten dough (Chapter 6). A key conclusion in Chapter 7 was that that reducible covalent bonds form between the wheat gluten proteins and the oat proteins creating a hybrid, oat-gluten protein network.

8.1.2. Wheat Gluten Protein Enrichment of Flour

As discussed in Section 2.9.1, wheat gluten can be added to wheat flour to improve the bread, cookie, and pasta making performance of the flour [25, 177, 191, 201-203]. This gluten enrichment influences the formation of the protein network and improves the performance of bread, cookie, and pasta doughs. There have been few studies undertaken on the addition of wheat gluten to flour from other cereals, seeds, and legumes (such as rice flour). A recent study by Oszvald et al. [73] found that the incorporation of wheat gluten into rice flour altered the mixing properties of the dough. No reasons were suggested for this.

As discussed in Section 7.1.3, the incorporation of other proteins into wheat flour (such as soy protein) also influences the formation of the gluten protein network in the dough. In summary, the dilution of the wheat gluten proteins with other proteins negatively affects the performance of bread, cookie, and pasta doughs [176, 198, 204]. Perez et al. [176] found that soy proteins had an adverse effect on the formation of the protein network during dough making due to both physical and bonding interactions between the gluten and soy proteins. Marorimbo et al. [198] found that the gluten and soy proteins interacted through physical and bonding interactions and that this negatively affected dough properties.

Research discussed in Chapter 6 using confocal scanning laser microscopy techniques showed that an insoluble protein network forms in oat-gluten dough. As shown in

Chapter 5 of this thesis the oat-gluten dough produced in the Al-Hakkak Process has visco-elastic properties similar to wheat flour dough. The functionality of the insoluble, hybrid, oat-gluten protein from the Al-Hakkak Process compared to the functionality of wheat gluten protein was not established.

8.2. Methodology

In these trials insoluble oat-gluten protein produced from a single batch of the Al-Hakkak Process was recycled and used in subsequent batches as shown in Figure 8.1. Various recycle ratios were used in the subsequent batches to assess the functionality of the oat-gluten protein compared to the wheat gluten protein normally used in the Al-Hakkak Process. Various substitution rates of oat-gluten protein in place of wheat gluten protein were used. In subsequent batches, the yield and purity of the second generation oat-gluten protein product from the Al-Hakkak Process was used as a measure the functionality of the recycled oat-gluten protein.

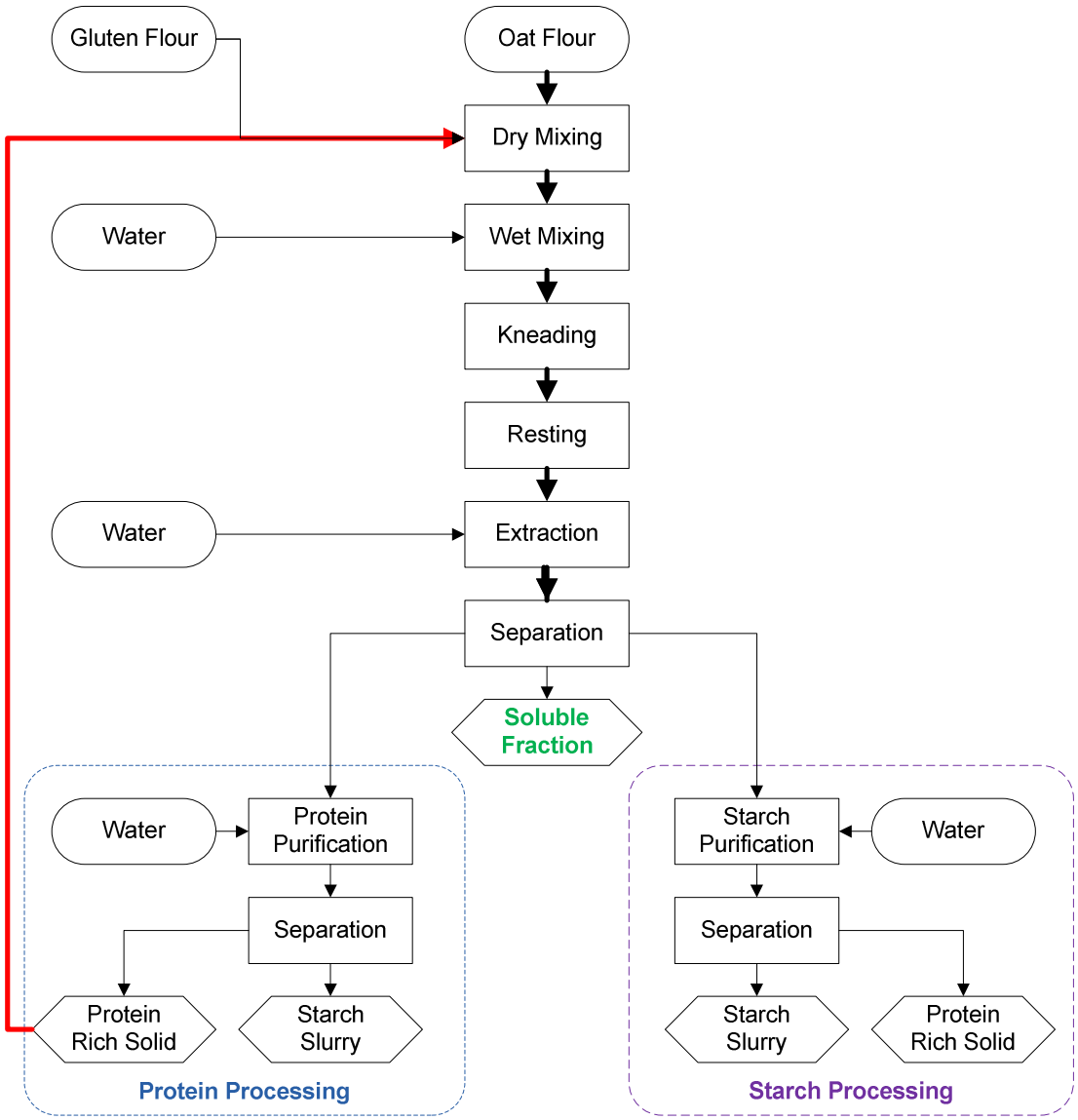


Figure 8.1. Schematic diagram showing the Al-Hakkak Process for these trials.

8.2.1. Equipment Selection

Samples were prepared using small pilot scale processing equipment that is similar in operation to large scale commercial processing equipment. Dry mixing, wet mixing, and kneading were carried out using a Farinograph mixer fitted with a 50 g kneading vessel as described in Section 3.2. Extraction was carried out using 500 ml stirred, baffled vessels, with a pitched blade impellor as described in Section 3.2.

8.2.2. Sample Preparation

Nine samples of the oat-gluten protein were prepared using the Al-Hakkak Process and substituting wheat gluten with various ratios of recycled insoluble oat-gluten protein. Table 8.1 summarises the experimental plan. The actual protein content of the wheat gluten flour, oat flour, and oat-gluten flour was accurately measured. The total protein content of each initial dough sample was calculated from this data.

Table 8.1: Experimental Plan – Dough Composition (estimated mass %)

Sample	Wheat gluten flour	Oat-gluten protein flour	Total protein
20/0 (standard)	20	0	20
0/20	0	20	20
0/30	0	30	30
0/40	0	40	40
10/10	10	10	20
10/20	10	20	30
10/30	10	30	40
15/5	15	5	20
15/25	15	25	40

Flour

Preparation and storage of the oat and gluten flour used in these trials is described in Section 3.3.

The oat-gluten protein flour was prepared using freeze dried oat-gluten protein from a single pilot scale Al-Hakkak Process batch (18th August 2009). The oat-gluten protein was frozen immediately following isolation and purification and then freeze dried. The freeze dried protein was in a granular form with a particle size of typically 5 mm to 10 mm. To reduce the particle size the oat-gluten protein granules were ground by hand using a pestle and mortar and then sieved using a 500 µm vibrating sieve. Particles greater than 500 µm were returned for further grinding. Samples of the oat-gluten protein flour were tested for total protein content.

Dough Processing

The nine dough samples were prepared using the Farinograph mixer using the recipes shown in Table 8.2. The dough was prepared by varying the standard recipe using various ratios of wheat gluten flour and oat-gluten flour. All other recipe conditions were held constant. All of the samples were produced using the same kneading conditions (Table 8.3). After kneading the samples were put into individual plastic bags, to minimise moisture loss during the resting period.

Table 8.2: Dough Recipe

Sample	**20/0 (standard)**	**0/20**	**0/30**	**0/40**
Oat flour (g)	48.10	45.90	43.80	41.90
Wheat gluten flour (g)	12.00	0.00	0.00	0.00
Oat-gluten protein flour (g)	0.00	14.10	16.20	18.10
2 % salt solution (NaCl) (g)	2.01	2.01	2.01	2.01
Water (g)	38.6	38.6	38.6	38.6
Water temperature (°C)	30.0	30.0	30.0	30.0

Sample	**10/10**	**10/20**	**10/30**	**15/5**	**15/25**
Oat flour (g)	48.10	43.70	40.10	48.10	40.10
Wheat gluten flour (g)	6.00	5.50	5.00	9.00	7.50
Oat-gluten protein flour (g)	5.90	10.80	14.80	3.00	12.40
2 % salt solution (NaCl) (g)	2.01	2.00	2.00	2.00	2.00
Water (g)	38.6	38.6	38.6	38.6	38.6
Water temperature (°C)	30.0	30.0	30.0	30.0	30.0

Table 8.3: Operating Conditions for Dough Preparation

Kneading temperature	°C	30
Wet kneading time	seconds	120
Resting temperature	°C	25
Resting time	minutes	90

Extraction and Purification

All nine samples were produced following the Al-Hakkak Process using the same extraction and purification protocols as described in Section 7.2.2. The specific operating conditions are summarised in Table 8.4.

Table 8.4: Operating Conditions for the Extraction Process

Dough mass washed	g	50.0
Water mass	g	200
Water temperature	°C	22
Extraction time	minutes	40

Gluten Protein Separation and Agglomeration Index

The oat-gluten protein agglomerates were separated from the extract liquor and starch granules using a sieving technique (i.e. a particle size basis) and the agglomeration index measured following the method described in Section 3.7.

8.2.3. Composition Analysis

The composition of the samples of second generation oat-gluten protein product from the Al-Hakkak Process were analysed to determine any differences between samples in the protein yield and composition as described in Section 3.10.

8.3. Results

8.3.1. Dough Composition

The protein contents of the gluten flour, oat-gluten protein flour, and oat flour from the Al-Hakkak Process were determined. The protein in the gluten flour was assumed to be 100 % gluten protein with no other protein fractions present. The gluten content of the oat-gluten flour was estimated by calculating the gluten contribution from the

original Al-Hakkak Process batch used to prepare the oat-gluten flour used in these trials (Table 8.5).

Table 8.5: Protein Content of Flours (mass %)

Flour sample	Total protein	Gluten protein
Oat flour	11.2	0.0
Gluten flour	81.6	81.6
Oat-gluten flour	48.4	31.2

The total protein content and gluten protein content of each dough sample prior to extraction was calculated based on initial flour composition (Table 8.6).

Table 8.6: Protein Content of Initial Dough Samples (mass %)

Sample	Total protein	Gluten protein	Oat protein
20/00	25.2	16.2	9.0
00/20	18.6	6.2	12.4
00/30	21.2	8.4	12.8
00/40	23.5	10.3	13.2
10/10	21.9	11.3	10.7
10/20	24.3	13.0	11.3
10/30	26.3	14.5	11.8
15/5	25.8	15.3	10.5
15/25	27.7	16.6	11.0

8.3.2. Protein Product Yield and Purity

The protein purity and yield of the second generation oat-gluten protein product (the protein rich solids recovered after sieving) was analysed (Table 8.7 and Figure 8.2). A comparison was also made of both the protein purity and yield of the second generation oat-gluten protein product compared to the initial dough composition.

Table 8.7: Protein Content of Recovered Oat-Gluten Protein Product (by mass) *

Sample	**Total mass recovered** (g)	**Total protein recovered** (g)	**Protein purity** (%)	**Protein yield** (%)
20/00	14.36	6.82	47.5	90
00/20	8.20	3.01	36.7	54
00/30	10.60	4.43	41.8	70
00/40	13.29	5.59	42.1	80
10/10	11.64	5.17	44.4	79
10/20	13.65	5.86	42.9	80
10/30	14.24	6.36	44.7	80
15/5	14.69	5.96	40.6	77
15/25	15.49	7.24	46.7	87

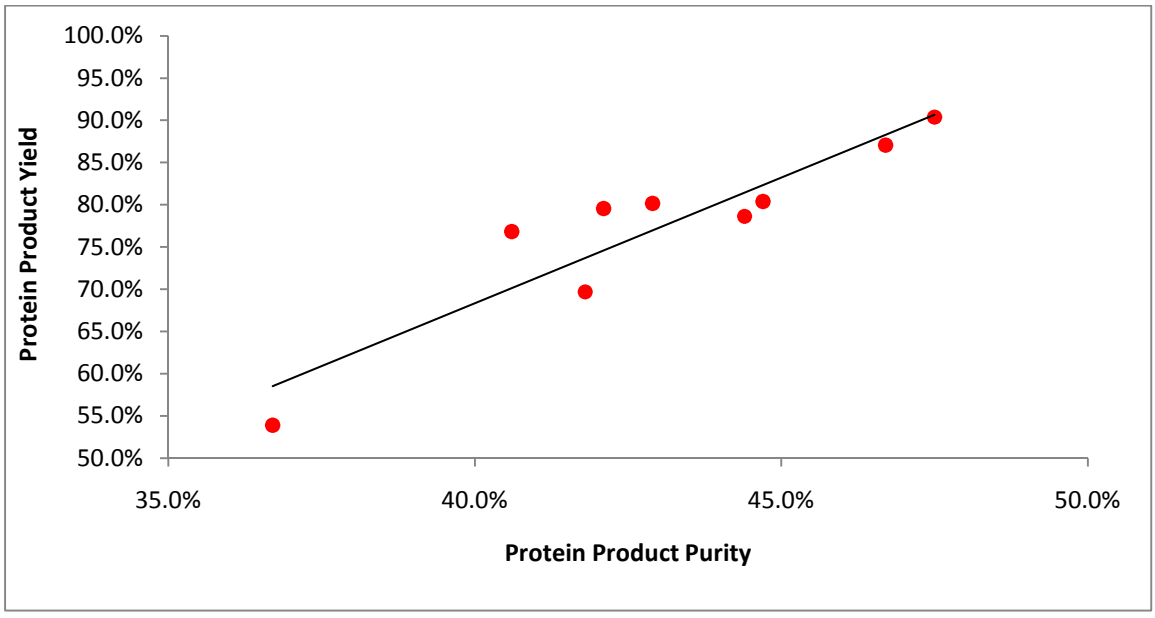


Figure 8.2. Comparison of the purity and yield of the recovered oat-gluten protein product.

Figure 8.2 shows a linear relationship between the second generation oat-gluten protein product yield and purity. From Table 8.7 and Figure 8.2 the following relationship between the purity and yield of recovered oat-gluten protein has been calculated:

$$YP = 2.98\,PP - 0.51 \quad (18)$$

$$R^2 = 0.855$$

where:

- YP = oat-gluten protein product yield (% mass of initial protein content of flour)
- PP = oat-gluten protein product purity (% mass)

Figures 8.3 to 8.6 show the relationship between the initial protein content of the dough and the protein yield and purity of the second generation oat-gluten protein product from the Al-Hakkak Process. Three general trends are observed in Figures 8.3 and 8.4. As the total protein and gluten protein content of the dough increases the protein product purity and yield from the Al-Hakkak Process generally increase. There is a decreasing trend in protein product purity and yield with increasing oat protein in the dough.

Figures 8.5 and 8.6 show the effect of substituting gluten flour with oat-gluten flour using four different substitution levels (0 %, 50 %, 75 %, and 100 % substitution). In general, as both the total protein and gluten protein content of the dough increase the protein product yield from the Al-Hakkak Process increases. The data points from the different dough samples sit on the same line.

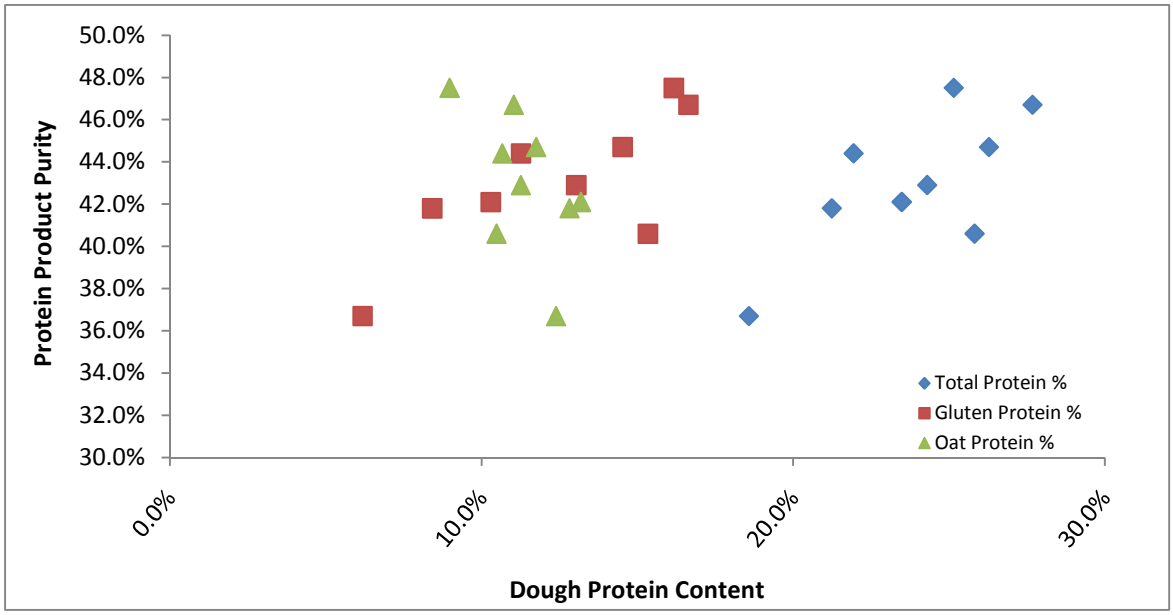


Figure 8.3. Comparison of the purity of the oat-gluten protein product with the content of gluten protein, oat protein, and total protein in the initial dough.

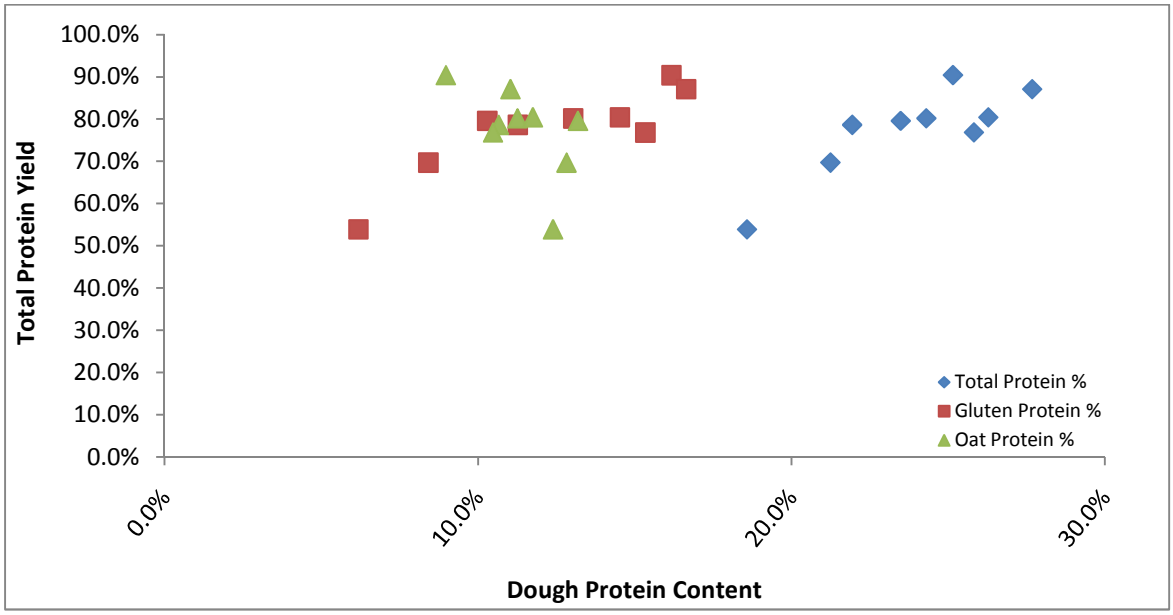


Figure 8.4. Comparison of the yield of the oat-gluten protein product with the content of gluten protein, oat protein, and total protein in the initial dough.

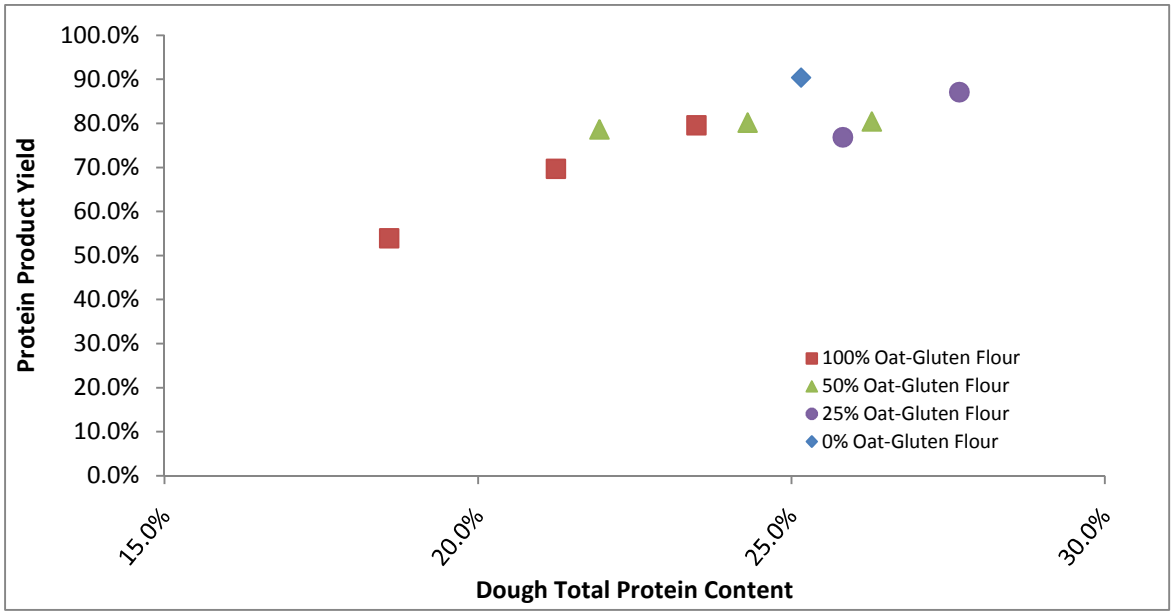


Figure 8.5. Comparison of the yield of the oat-gluten protein product with the content of total protein in the initial dough for 0 %, 50 %, 75 % and 100 % substitution of gluten flour with oat-gluten flour.

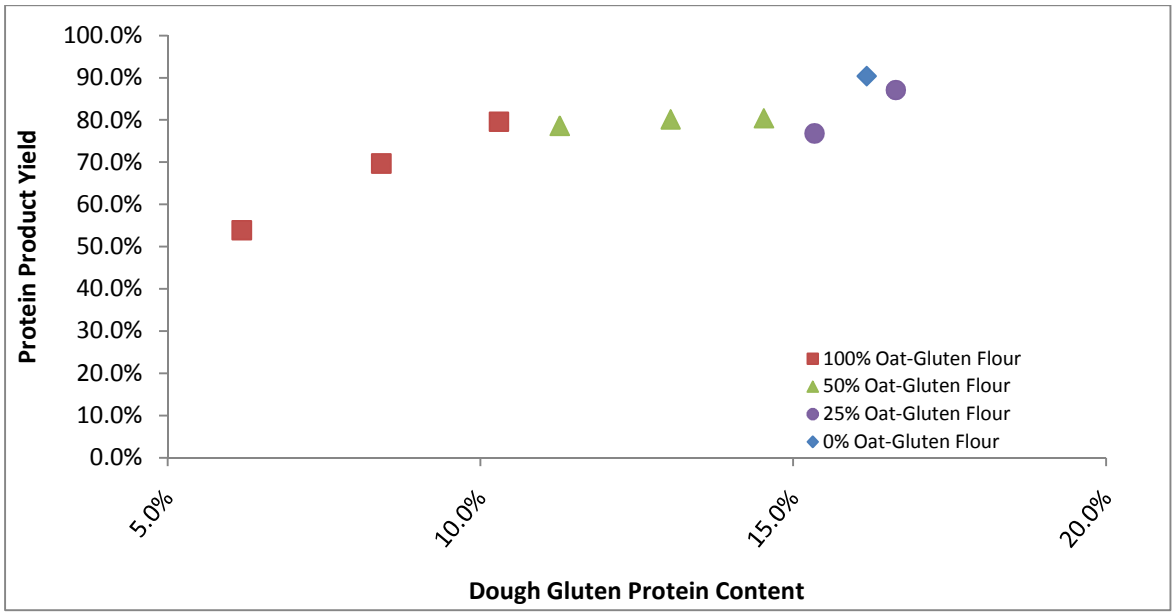


Figure 8.6. Comparison of the yield of the oat-gluten protein product with the content of gluten protein in the initial dough for 0 %, 50 %, 75 % and 100 % substitution of gluten flour with oat-gluten flour.

8.3.3. Recovered Oat-Gluten Protein Product Agglomerate Size

Sieving is commonly used to assess the gluten protein agglomeration in wheat gluten separation processes (such as the Martin Process and the Batter Process) [25]. Poor agglomeration produces a protein network consisting of smaller protein particles which can pass through the 400 µm, whereas good agglomeration produces large protein particles which are retained. The gluten agglomeration index is measured by comparing the mass of protein retained by the 400 µm sieve with the combined protein recovered from 400 µm and 125 µm sieves. This method was applied to the second generation oat-gluten protein product from the Al-Hakkak Process.

The mass fractions of the agglomerates of oat-gluten protein product that make up the cohesive protein network in dough was measured and the results are shown in Table 8.8.

Table 8.8: Protein Particle Size (mass %)

Sample	**Solids mass fraction >400 µm** (±1.0 %)	**Solids mass fraction <400 µm and >125 µm** (±1.0 %)
20/00	96	4
00/20	85	15
00/30	90	10
00/40	97	3
10/10	93	7
10/20	94	6
10/30	95	5
15/5	96	4
15/25	96	4

A comparison was made between the mass of second generation oat-gluten protein retained by the 400 µm sieve and both the oat-gluten protein purity and yield of the oat-gluten protein product (Figures 8.7 and 8.8). Generally as the oat-gluten protein purity and yield increased, so did the second generation oat-gluten protein mass retained by the 400 µm sieve. The relationship between the mass of second generation oat-gluten protein retained by the 400 µm sieve and the initial protein content of the dough (both total protein and gluten protein) was also investigated (Figures 8.9 and

8.10). Generally, increasing the total protein and gluten protein content of the initial dough resulted in an increase in the second generation oat-gluten protein retained by the 400 µm sieve.

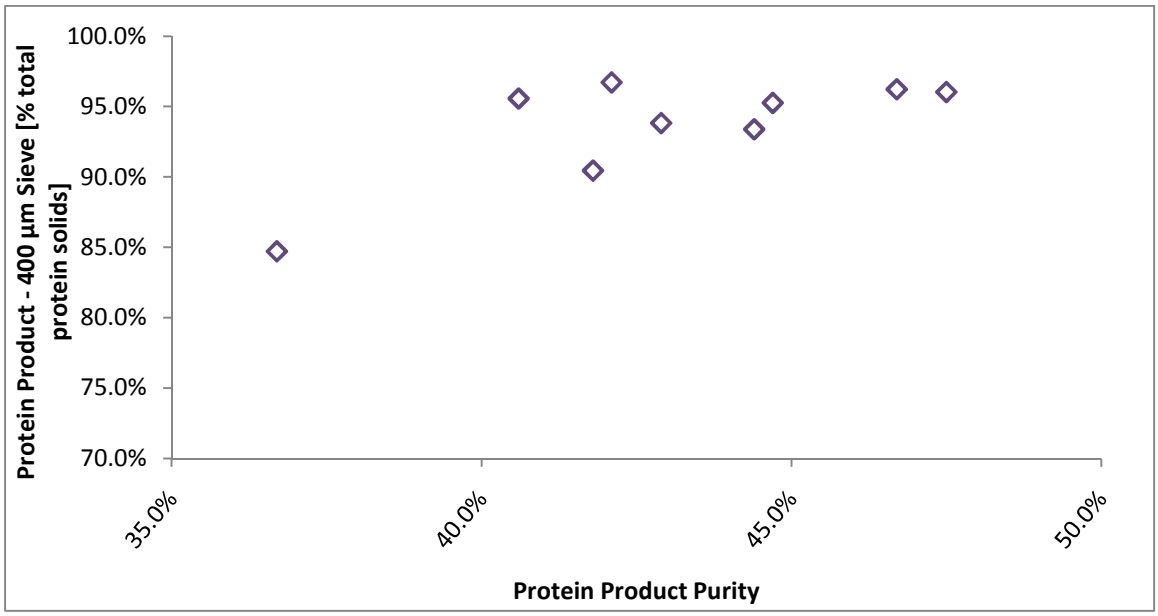


Figure 8.7. Comparison between the oat-gluten protein retained by the 400 µm sieve and the purity of the oat-gluten protein product.

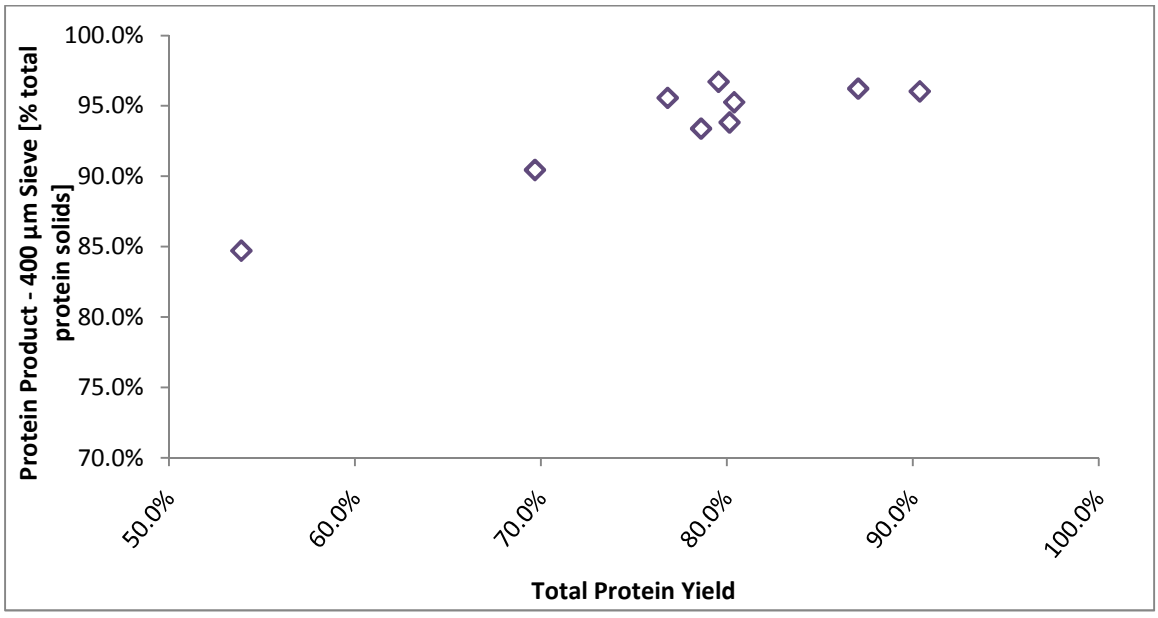


Figure 8.8. Comparison between the oat-gluten protein retained by the 400 µm sieve and the yield of the oat-gluten protein product.

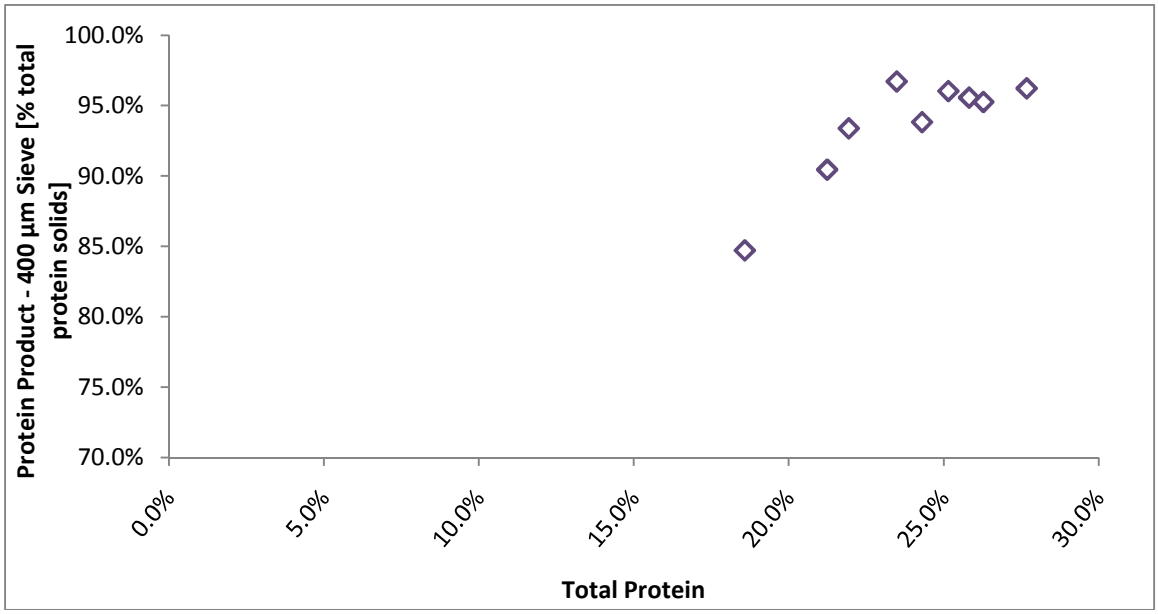


Figure 8.9. Comparison between the oat-gluten protein retained by the 400 µm sieve and the total protein content of the initial dough.

Figure 8.10. Comparison between the oat-gluten protein retained by the 400 µm sieve and the gluten protein content of the initial dough.

8.4. Discussion

The Al-Hakkak Process generates two main co-products, protein and starch. Protein yield and purity are important processing parameters as they provide information on the starch and protein separation processes. Protein yield and purity can be used to measure the effectiveness of starch and protein separation. Low protein purity would indicate that there was poor separation of the starch and protein. As a result a proportion of the starch product would be lost with the protein stream, reducing the mass of starch produced per kilogram of flour. The lost starch would contaminate the protein product stream, potentially reducing its value. These factors would negatively impact on the economics of commercial production. Low protein yield would indicate that protein was lost, most likely to the starch stream reducing the starch purity. Certain processing conditions can increase the solubility of the protein stream resulting in protein lost in the soluble fraction (the supernatant stream). As discussed at the start of this chapter cereal proteins are a valuable and any loss would negatively impact on the economics of commercial production. Starch purification processes are already well established in the starch industry, using technologies such as sedimentation, centrifugation, and screening (these processes have already been discussed in more detail in Section 2.9.4 of this thesis). The starch co-product stream from the Al-Hakkak Process could be readily purified using these existing technologies.

8.4.1. Flour

The total protein content of the oat-gluten protein flour was less than 50 %, due to starch contamination (Table 8.5). This low purity was expected, as the protein samples were taken after the initial extraction step prior to protein purification. Calculations identified that 64.4 % of the protein in the oat-gluten protein flour was wheat gluten and the remainder of the protein was insoluble oat protein. In an optimised Al-Hakkak Process the protein concentration of the recycled oat-gluten protein used to make the oat-gluten protein flour would be higher. This is because oat-gluten protein would be processed through several purification steps prior to use.

The combined mass fraction oat-gluten protein flour and gluten flour was varied from 20 % to 40 % of the total flour. The total protein content of the dough ranged from 18.6 % to 27.7 % (Table 8.6). The gluten protein fraction came from both the oat-gluten protein flour and the gluten flour. The gluten content ranged from 6.2 % to 16.6 % of the dough (by mass) and from 33.3 % to 64.3 % of the total protein in the dough (Table 8.6). Oat proteins comprised the remainder of the dough protein. Previous studies on the Al-Hakkak Process have shown that adding 14.4 % gluten protein (18 % of a commercial gluten flour at 80 % purity) to oat flour produced starch co-product of acceptable yield and purity [51, 52]. No information was reported on the protein purity of the oat-gluten protein product stream. The range of total protein and gluten protein concentrations investigated in the trials undertaken as part of this study aligned well with the gluten protein concentrations used in the previous study on the Al-Hakkak Process.

8.4.2. Oat-Gluten Protein Product Yield and Purity

Figure 8.2 and Table 8.7 show that, as the second generation oat-gluten protein product yield (as a percentage of the initial protein content) from the Al-Hakkak Process increased, the protein purity also increased. The relationship between oat-gluten protein product yield and purity was closely correlated (R^2 = 0.855) and appeared linear. Hence, there was no optimum oat-gluten protein yield or purity target and any improvement in oat-gluten protein yield also improved oat-gluten protein purity. This is important information for commercial manufacturing.

A greater total protein content in the initial dough resulted in an increase in both the yield and purity of the second generation oat-gluten protein product from the Al-Hakkak Process (Figures 8.4 and 8.5). A similar trend was displayed between the gluten protein content of the initial dough and the oat-gluten protein product yield and purity. The relationship appeared linear. This supports the hypothesis that the gluten protein promoted the formation of the large protein particles that make up the cohesive protein network in the dough. Both the total protein and gluten protein content of the initial dough were good indicators of the oat-gluten protein yield and purity from the Al-Hakkak Process.

Increasing oat protein content resulted in a slight decrease in the yield and purity of the second generation oat-gluten protein product. In other studies the deleterious influence of non-gluten proteins in the formation of the protein network in dough has been attributed to the dilution of the gluten proteins [176]. The results of this study are consistent with this, suggesting that the oat protein slightly diluted the functionality of the gluten protein. The negative effect of the oat protein content on yield and purity is not as pronounced as the positive influence of the gluten protein. This suggests that, whilst the gluten proteins are a key factor the formation of the protein particles, the oat proteins are also involved in the protein agglomerate formation.

For any given substitution of gluten flour with oat-gluten protein flour (25 %, 50 %, and 100 %) the yield of second generation oat-gluten protein product increased with both increasing total protein content or gluten protein content of the initial flour (Figures 8.6 and 8.7). This indicates that gluten content of the oat-gluten flour is the key factor for the formation of the oat-gluten protein network.

As discussed earlier in this chapter, wheat gluten proteins interact and agglomerate to form a cohesive, visco-elastic, protein network during dough kneading [25, 72, 78, 174, 176]. The formation of the gluten protein network is due to the formation of disulphide bonds as well as other covalent and non-covalent bonds between individual protein molecules [6, 34, 70, 73, 176, 192]. During kneading these inter-molecular bonds break and are reformed between different gluten protein molecules. This ability of the gluten proteins to allow the inter-molecular protein bonds to break and reform is exploited in bread, baking, and pasta making where gluten enrichment is used to improve the dough [25, 177, 191, 201-203].

Increasing both the total protein and gluten protein content of the initial dough resulted in an increase in both the protein yield and purity of the second generation oat-gluten protein product. This suggests that the source of the gluten proteins (either gluten flour or from oat-gluten flour recycled from a previous Al-Hakkak Process batch) did not alter the functionality of the gluten proteins. Thus, the oat-gluten protein flour from the Al-Hakkak Process can be used to replace commercial gluten flour. However, the recycled oat-gluten protein is diluted by oat protein and has a lower gluten protein

content compared to commercial gluten flour. Therefore, compared to gluten flour, a greater mass of oat-gluten protein is needed to achieve the same degree of protein network-forming functionality.

In commercial wheat gluten separation processes (such as the Martin Process and Batter Process) the percentage of protein recovered from the flour varies from 72 % to 92 % [25, 60]. These trials have shown that for the Al-Hakkak Process the best oat-gluten protein yield of greater than 87 % (Samples 20/10 and 15/25) was achieved when there was more than 16.1 % gluten protein in the initial dough sample. Good oat-gluten protein product yields of greater than 77 % (Samples 0/40, 10/10, 10/20, 10/30, and 15/5) were achieved when there was more than 11.3 % gluten protein in the initial dough sample. This compares with previous studies investigating the purity of the starch co-product from the Al-Hakkak Process, which found that 14.4 % gluten protein was optimal [51, 52]. This is important information for optimising the economics of a commercial manufacturing process. The total protein and gluten protein content of the initial dough can be used to control the oat-gluten protein product yield and purity.

8.4.3. Protein Agglomerate Size

For all of the samples, the majority of the second generation oat-gluten protein product was retained by the 400 µm sieve, with only a small amount recovered from the 125 µm sieve (Table 8.8). Similar results have been reported in other studies on the separation of gluten in wheat dough [180].

Figures 8.8 and 8.9 show that, the greater the proportion of oat-gluten protein product recovered on the 400 µm sieve, the greater the second generation oat-gluten protein product yield and purity from the Al-Hakkak Process. This suggests that larger oat-gluten agglomerates produced greater yield with higher purity. Generally a greater total protein content in the initial dough resulted in an increase in the proportion of second generation oat-gluten protein recovered on the 400 µm sieve (Figure 8.9). A similar trend was observed with increasing gluten content in the initial dough (Figure 8.10). This indicates that the protein content of the initial dough is a key factor in the formation of large oat-gluten protein agglomerates. This is important information for

optimising a commercial manufacturing process as the total protein and gluten protein of the initial dough content can be easily controlled.

8.5. Conclusions

A key conclusion from this research was that the source of the gluten proteins (either the added gluten flour or from oat-gluten protein flour recycled from a previous Al-Hakkak Process batch) did not alter their functionality. Hence, oat-gluten protein flour from the Al-Hakkak Process could be recycled and used to replace the commercial gluten flour ingredient in subsequent Al-Hakkak Process batches. This confirmed the hypothesis stated at the start of this Chapter. However, the oat proteins present in the oat-gluten protein from the Al-Hakkak Process were shown to have a diluting effect on the protein network functionality of the gluten protein. Hence, a greater mass of oat-gluten protein flour was required to achieve the same degree of protein network formation as achieved using gluten flour.

Increasing the gluten protein content of the initial oat-gluten dough promoted the formation of large second generation oat-gluten protein agglomerates. It was concluded that the gluten is the source of the protein network-forming functionality of the oat-gluten protein. Oat protein present in the initial dough had a slightly deleterious effect on the functionality of the second generation oat-gluten protein and it was concluded that this was because of the dilution of the gluten proteins with oat proteins.

It was concluded that the oat proteins were involved in the formation of oat-gluten protein network during the Al-Hakkak Process. Whether this interaction is due to chemical bonding (covalent, ionic, or other) or entanglement was not investigated. This has been the focus of investigations described in Chapters 6 and 7 of this thesis which showed that chemical bonding occurs between the oat and gluten proteins. Whether entanglement of the proteins also occurs has not been established, but this is considered likely.

Important information for optimising the economics of a commercial manufacturing process was identified. The protein content (both total protein and gluten protein) of the initial dough were found to be a good indicator of both the oat-gluten protein yield and purity from the Al-Hakkak Process. The total protein and gluten protein of the initial dough content can be easily controlled. This study has shown that by varying these parameters, the yield, purity and particle size of the second generation oat-gluten protein product from the Al-Hakkak Process can be controlled.

9. Al-Hakkak Process Variability

9.1. Introduction

This chapter discusses the effects of altering various Al-Hakkak Process parameters (processing and composition of dough) on the hybrid oat-gluten protein yield and purity [51, 52]. It brings together the knowledge established and reported elsewhere in this thesis on the formation, structure and functionality of the hybrid oat-gluten protein network.

9.2. Background

9.2.1. The Al-Hakkak Process

As discussed in Section 2.11 of this thesis, the Al-Hakkak Process is similar to the traditional Martin Process for wheat gluten and starch separation. It involves the three key processing steps 1) oat-gluten dough kneading and development, 2) aqueous extraction, and 3) oat-gluten protein and starch separation. It is likely that operating conditions used in each of these processes influences the final purity and yield of the hybrid oat-gluten protein product as well as the starch co-product. The focus of this study has been to investigate key processing parameters for the Al-Hakkak Process.

An earlier study had been undertaken into the separation of protein and starch using the Al-Hakkak Process [51]. This study investigated the performance of the Al-Hakkak Process using several different raw materials, including oat. Whilst the study was broad in terms of raw material feedstock, the focus was narrow in terms of separation performance. The results of this earlier study focused entirely on the purity of the starch co-product stream. The yield and purity of the insoluble oat-gluten protein product stream was not investigated. The effect on the starch co-product stream of varying the gluten content, sodium chloride concentration, and ascorbic acid concentration of the dough on the purity was investigated. The influences of other processing parameters on the performance of the Al-Hakkak Process were not

considered (for example: kneading time, resting time, extract liquor temperature, and extract liquor pH).

9.2.2. These Investigations

The hypothesis for these trials was that varying the initial dough composition and subsequent dough and extraction processing parameters affects the formation of the oat-gluten protein network. This, in turn, influences the separation of starch and protein during the Al-Hakkak Process. Thus, an optimal set of processing conditions exists for the Al-Hakkak Process.

The aim of this part of the project was to investigate the effect of varying processing parameters and dough composition on the separation of oat-gluten protein and starch using the Al-Hakkak Process. The focus of the trials was the degree of agglomeration of the insoluble oat-gluten protein product. This was assessed by measuring the yield and purity of the oat-gluten protein fraction retained by vibrating screens of decreasing aperture size. Comparisons were made between these results and the results and conclusions made earlier in this thesis.

9.3. Methodology

The oat-gluten protein samples in these trials were prepared using the Al-Hakkak Process [51, 52]. Samples were taken of the agglomerated oat-gluten protein after the initial separation stage, prior to subsequent purification to that the effects of kneading and extraction could be assessed (Figure 9.1).

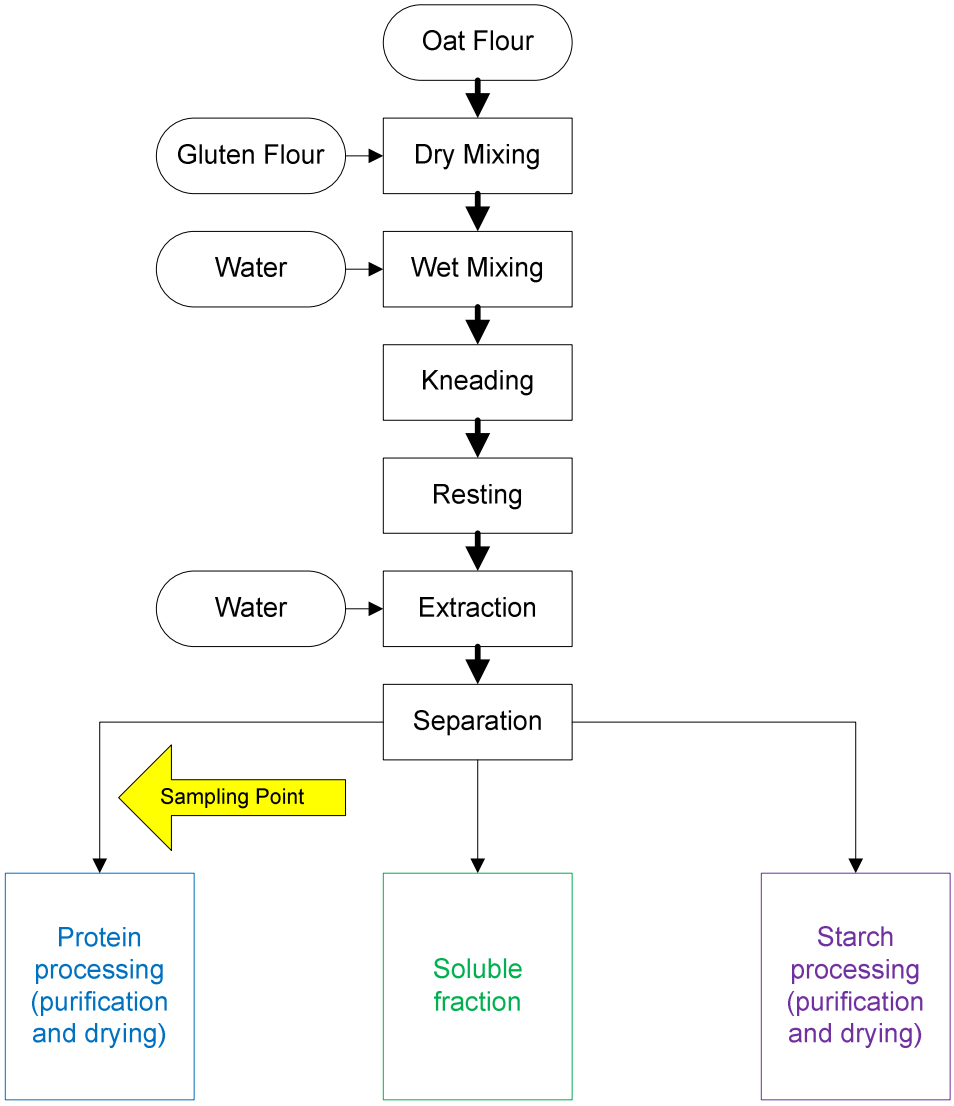


Figure 9.1. Schematic diagram showing the Al-Hakkak Process for these trials.

9.3.1. Equipment Selection

Samples were prepared using two scales of processing equipment that were similar in operation to large scale commercial processing equipment. Small pilot scale dry mixing, wet mixing, and kneading was carried out using a Farinograph mixer fitted with a 50 g kneading vessel. The larger pilot scale dry mixing, wet mixing, and kneading was carried out using a small, two speed, dough mixer fitted with

approximately 5 kg capacity vessel and a single dough hook (“E” dough arm). Both are described in Section 3.2. Extraction was carried out using 500 ml stirred, baffled vessels, with a pitched blade impellor as described in Section 3.2.

9.3.2. Sample Specification

The effect of varying processing parameters relating to dough production and extraction processes was investigated. Several operating conditions were considered:

1. Sodium chloride content of the oat-gluten dough and extract liquor
2. Gluten content of the oat-gluten dough
3. Kneading time
4. Processing scale (small and large pilot scale)
5. Extraction temperature
6. Extract liquor pH

Table 9.1 summarises the samples prepared for these trials. Details of the processing conditions used for each sample are presented later in this section.

Table 9.1: Experimental Plan

Sample Identification	Description
1	Standard conditions – small pilot scale
B	Oat dough
C	Gluten-starch dough
3	Short dough kneading
2	Long dough kneading
12	Very long dough kneading
4	Low sodium chloride content in dough
5	Very low sodium chloride content in dough
7	Low gluten content in dough
8	High gluten content in dough
A	Standard conditions – large pilot scale
22	Extract liquor pH 2.7
23	Extract liquor pH 2.0
24	Extract liquor temperature T = 15 °C
25	Extract liquor temperature T = 30 °C
26	Extract liquor temperature T = 40 °C
27	Extract liquor temperature T = 50 °C
28	1 % sodium chloride content in extract liquor
29	2 % sodium chloride content in extract liquor

9.3.3. Sample Preparation

All of the samples were oat-gluten protein agglomerates taken following the initial extraction and separation stage of the Al-Hakkak Process (as shown in Figure 9.1), but before subsequent processing to purify the protein by removing residual starch granules still trapped in the oat-gluten protein network. This sample location was selected as it would provide information on the dough production and extraction processes that could be compared with other results from this study. Purification processes involve mixing and agitation. Other research undertaken in this study (Chapters 5 and 6) has shown that such processes influence the formation oat-gluten protein network in the Al-Hakkak Process (see Section 2.11). Hence, the selection of

the sampling point eliminates any possible modification of the oat-gluten agglomerates due to purification processes.

Flour

Preparation and storage of the flour used in these trials is described in Section 3.3.

Only oat flour was used to prepare Sample B (i.e. no gluten flour was used). This was to investigate the natural size of the insoluble oat protein particles in the Al-Hakkak Process without gluten protein promoting protein agglomeration. Sample C was produced using gluten flour and starch to create a "manufactured" wheat flour composition. This was to investigate the performance of the Al-Hakkak Process without oat protein present. It is widely accepted that different wheat flours perform differently in gluten and starch separation processes [25, 59, 63, 64]. This is due in part to the gluten proteins having variable agglomeration functionality depending on a range of factors (for example: cultivar, season, and location). Hence, it was considered important to use the same gluten flour as was used for the other samples to eliminate this potentially complicating factor.

Kneading and Resting

Both the dough composition and processing conditions were expected to influence the yield and purity of the oat-gluten protein produced by the Al-Hakkak Process. Hence, the effect of changing various processing parameters was investigated. Each small pilot scale sample was produced using the Farinograph with an initial charge of 48.10 g oat flour as shown in Table 9.2. The large pilot scale samples were prepared using the Hobart mixing and an initial charge of 480.6 g as shown in Table 9.3.

Table 9.2: Oat-Gluten Dough Recipes – Small Pilot Scale

Sample description		**Standard**	**Low NaCl**	**No NaCl**	**Low gluten**	**High gluten**
Sample		1, 2, 3, 12	4	5	7	8
Oat flour	g	48.1	48.1	48.1	51.0	45.0
Gluten flour	g	11.9	11.9	11.9	9.0	15.0
2 % NaCl solution	g	2.0	1.0	0.0	2.0	2.0
Water	g	38.6	39.6	40.6	38.6	38.6
Water temperature	°C	30	30	30	30	30

Parameter		**Oat dough**	**Gluten-starch dough**
Sample		B	C
Oat flour	g	60.00	0.00
Wheat starch	g	0.00	48.10
Gluten flour	g	0.00	11.90
2 % NaCl solution	g	2.0	2.0
Water	°C	38.6	38.6
Water temperature		30	30

Table 9.3: Oat-Gluten Dough Recipe – Large Pilot Scale

Sample description		**Pilot scale**
Sample		A, 22 to 29
Oat flour	g	480.6
Gluten flour	g	119.4
2 % NaCl solution	g	20.1
Water	g	386.4
Water temperature	°C	30

The operating conditions used in the Al-Hakkak Process to produce oat-gluten dough varied between samples. Tables 9.4 and 9.5 show the operating conditions used to produce the oat-gluten dough at small and large pilot scale.

Table 9.4: Oat-Gluten Dough Processing – Small Pilot Scale

Sample description		Standard	Short kneading	Long kneading	Very long kneading
Sample		1, 4, 5, 7, 8	3	2	12
Kneading temperature	°C	30	30	30	30
Kneading time [a, b]	minutes	2	1.5	2.5	3.0
Resting temperature	°C	22	22	22	22
Resting time	minutes	90	90	90	90

a) Other research has identified that two minutes kneading in the small scale Farinograph is equivalent to ten minutes kneading in the pilot scale Hobart mixer.
b) Excludes 30 seconds dry mixing prior to water and sodium chloride solution addition.

Table 9.5: Oat-Gluten Dough Processing – Large Pilot Scale

Sample description		Pilot scale
Sample		A, 22 to 29
Kneading temperature	°C	30
Kneading time [a, b]	minutes	10
Resting temperature	°C	22
Resting time	minutes	90

a) Excludes 30 seconds dry mixing prior to water and sodium chloride solution addition.
b) The Hobart mixer (AE200) used in this trial has three speed settings. The slow setting (43rpm) was used for the first four minutes to allow the flour and water to form a cohesive oat-gluten dough. Then the second setting (150 rpm) was used for the remaining six minutes mixing time. The third setting was not used.

Extraction

Extraction conditions were also expected to influence the yield and purity of the oat-gluten protein produced by the Al-Hakkak Process. Hence, the effect of extraction time on the oat-gluten protein structure was investigated. Small pilot scale extraction was carried out on all samples. Tables 9.6 and 9.7 describe the operating conditions used for the initial extraction of the oat-gluten dough to separate the oat starch granules from the insoluble oat-gluten protein network.

Water at 22 °C was placed into the small scale extraction vessel and agitator positioned to the correct height (20 mm off the bottom of the vessel). The agitator was then turned on and the correct speed selected (corresponding to 120 rpm). A 50.0 g sample of dough was then cut into five pieces of similar size using hand scissors. These pieces

were then dropped individually over a period of about 20 seconds into the agitated water.

Table 9.6: Operating Conditions for the Extraction Process

Sample Description		Standard	Low pH	Very low pH	High NaCl	Very high NaCl
Sample		Standard	22	23	24	25
Dough mass	g	50.0	50.0	50.0	50.0	50.0
Water mass	g	200	200	200	200	200
Water temperature	°C	22	22	22	22	22
Extraction time	minutes	60	60	60	60	60
Extraction pH		3.9	2.7	2.2	3.9	3.9
NaCl content		0 %	0 %	0 %	1 %	2 %

Table 9.7: Operating Conditions for the Extraction Process – Temperature Samples

Sample Description		Cold	Warm	Hot	Very hot
Sample		24	25	26	27
Dough mass	g	50.0	50.0	50.0	50.0
Water mass	g	200	200	200	200
Water temperature	°C	15	30	40	50
Extraction time	minutes	60	60	60	60
Extraction pH		3.9	3.9	3.9	3.9
NaCl content		0 %	0 %	0 %	0 %

Gluten Protein Separation and Agglomeration Index

The agglomerates of oat-gluten protein were separated from the extract liquor and starch granule slurry using a sieving technique (i.e. a particle size basis) and the agglomeration index measured following the method described in Section 3.7.

9.3.4. Composition Analysis

The composition of the samples of oat-gluten protein were analysed to determine any differences between samples in the protein yield and composition as described in Section 3.10.

9.4. Results

9.4.1. Calculations

From the experimental raw data collected a number of parameters were calculated to assess the performance of the Al-Hakkak Process.

- **Total solids yield.** Mass percent of total solids (dry) collected on the 400 µm sieve, 125 µm sieve, or 400 µm and 125 µm sieves, relative to the initial flour mass (dry).
    - TS_{400} = Total solids yield collected on the 400 µm sieve
    - TS_{125} = Total solids yield collected on the 125 µm sieve
    - TS = Total solids yield collected on the 400 µm and 125 µm sieves
- **Oat-gluten protein product yield.** Mass percent of protein (dry) collected on either: the 400 µm sieve, 125 µm sieve, or 400 µm and 125 µm sieves relative to the protein content of the initial flour (dry).
    - YP_{400} = Oat-gluten protein product yield collected on the 400 µm sieve
    - YP_{125} = Oat-gluten protein product yield collected on the 125 µm sieve
    - YP = Oat-gluten protein product yield collected on the 400 µm and 125 µm sieves
- **Oat-gluten protein product purity.** The mass percent of protein (dry) collected on either: the 400 µm sieve, 125 µm sieve, or 400 and 125 µm sieves relative to the mass of total solids recovered on the respective sieve (dry).
    - PP_{400} = Oat-gluten protein product purity collected on the 400 µm sieve
    - PP_{125} = Oat-gluten protein product purity collected on the 125 µm sieve

- PP = Overall oat-gluten protein product purity collected on the 400 µm and 125 µm sieves

- **Protein agglomeration index.** Mass of protein product collected on the 400 µm sieve divided by the total mass of protein collected from both the 400 µm and 125 µm sieves (dry).

9.4.2. Images of Sieving

A comparative and qualitative assessment was undertaken comparing the consistency of the separated protein produced by the Al-Hakkak Processing using oat-gluten flour, oat flour, and gluten-starch flour. The protein collected on the 400 µm sieve was compared and photographed oat-gluten flour (Sample 1), oat flour (Sample B), and gluten-starch flour (Sample C) using standard processing parameters. Sample C (Figure 9.4) formed a single, cohesive mass on the 400 µm sieve which had a rubbery consistency. Sample 1 (Figure 9.2) formed a single, cohesive mass on the sieve which was pale brown in colour. However, this protein mass was looser (spreading out more over the surface of the sieve) and had a softer consistency. Sample B (Figure 9.3) did not form a cohesive mass, but formed a slurry formed across the surface of the sieve that could be scraped into a mound using a spatula. This slurry was brown in colour and had a consistency similar to watery porridge.

Figure 9.2. The consistency of the oat-gluten protein product produced using oat-gluten flour (Sample 1).

Figure 9.3. The consistency of the oat protein product produced using oat flour (Sample B).

Figure 9.4. The consistency of the gluten protein product produced using gluten-starch flour (Sample C).

9.4.3. Overall Results

The dry mass and protein content of the oat-gluten protein collected on the 400 µm sieve and the 125 µm sieve was measured. From this data the oat-gluten protein yield and purity was calculated for each sample as shown in Table 9.8. The effects of the individual parameters are presented in the later section.

Table 9.8: Total Protein Content of Recovered Oat-Gluten Protein Product (% dry by mass)

Sample	400 µm sieve		125 µm sieve	
	Oat-gluten protein purity [a]	Oat-gluten protein yield [b]	Oat-gluten protein purity [a]	Oat-gluten protein yield [b]
1	42.2 %	80 %	44.2 %	4 %
B	22.6 %	14 %	22.0 %	7 %
C	64.4 %	100 %	-	-
3	38.7 %	71 %	43.0 %	5 %
2	41.8 %	74 %	41.6 %	5 %
12	39.8 %	81 %	44.4 %	6 %
4	43.7 %	80 %	46.1 %	6 %
5	40.6 %	71 %	43.3 %	6 %
7	40.5 %	66 %	37.0 %	9 %
8	45.7 %	83 %	44.3 %	4 %
A	50.8 %	78 %	41.3 %	4 %
22	49.0 %	84 %	36.1 %	4 %
23	46.4 %	86 %	36.9 %	3 %
24	44.9 %	82 %	33.9 %	3 %
25	47.7 %	82 %	36.4 %	3 %
26	50.3 %	86 %	35.4 %	3 %
27	43.6 %	74 %	34.6 %	3 %
28	48.4 %	85 %	36.2 %	3 %
29	42.3 %	74 %	32.1 %	3 %

a) Purity is ± 0.5 %
b) Yield is ± 1.0 %

The relative contribution to the total oat-gluten protein yield from the material collected on the 400 µm sieve and 125 µm sieve is presented in Figures 9.5 and 9.6. These figures show that the majority of the oat-gluten protein solids were recovered on the 400 µm sieve with very little collected on the 125 µm sieve.

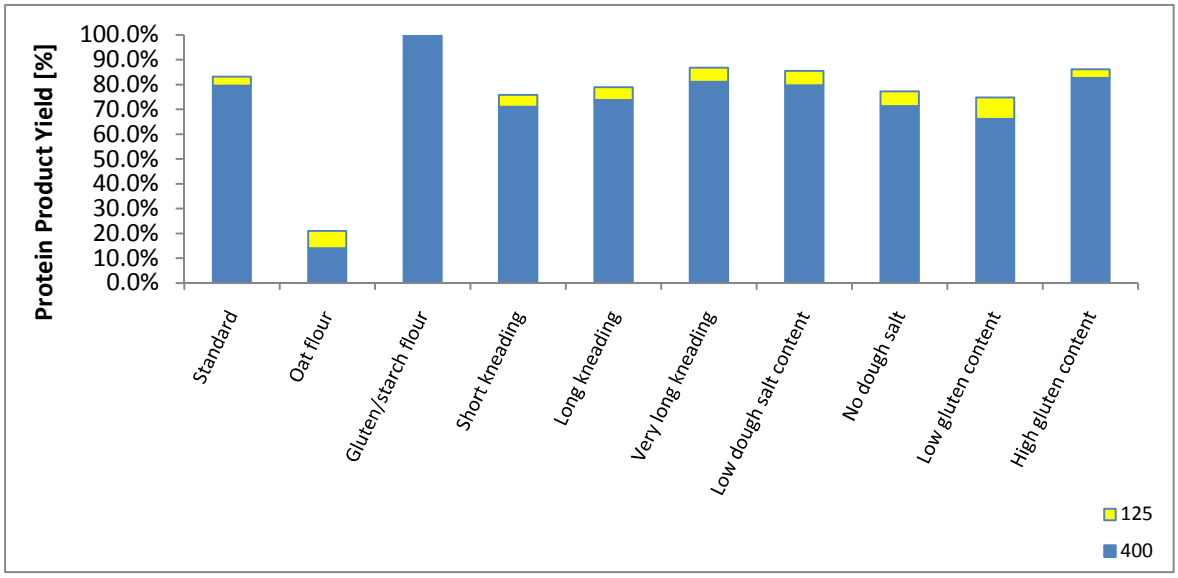


Figure 9.5. Oat-gluten protein yield collected on the 400 µm sieve and the 125 µm sieve for samples with varying dough composition.

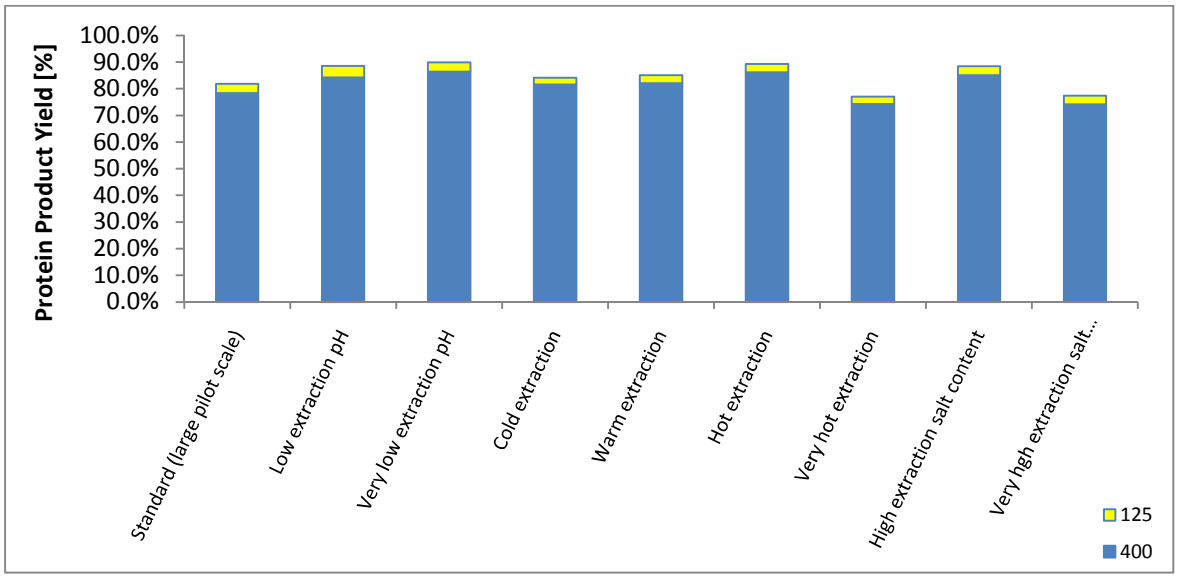


Figure 9.6. Oat-gluten protein yield collected on the 400 µm sieve and the 125 µm sieve for samples with varying extraction conditions.

A comparison of the oat-gluten protein purity of the material collected on the 400 µm and 125 µm sieves is presented in Figures 9.7 and 9.8. From this data the oat-gluten protein agglomeration index was calculated for each sample (Table 9.9).

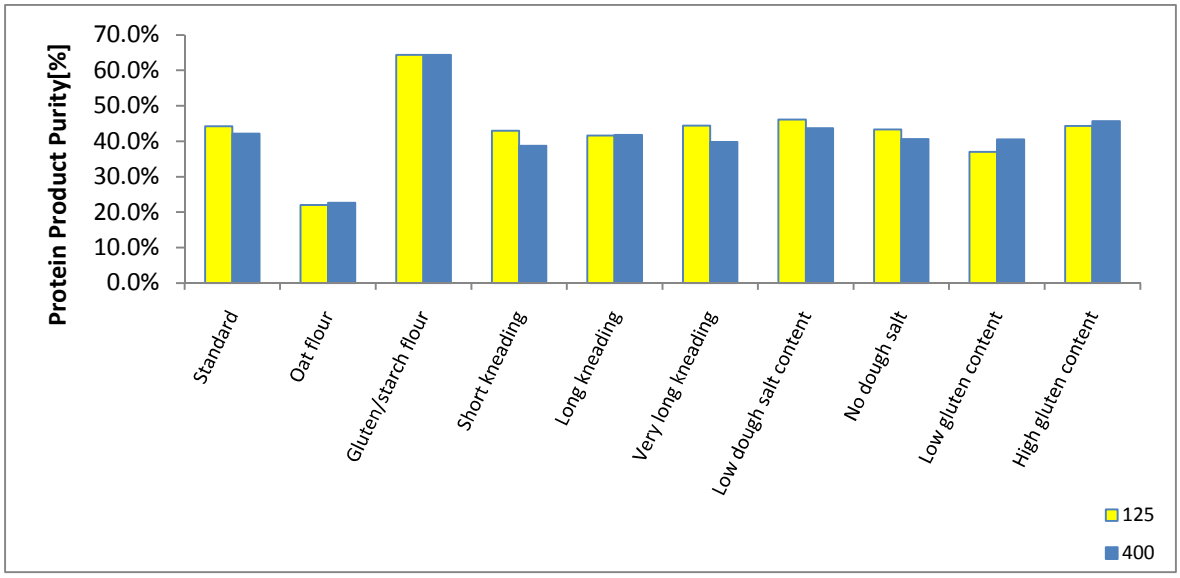


Figure 9.7. Oat-gluten protein purity of samples collected on the 400 µm sieve and the 125 µm sieve for samples with varying dough composition.

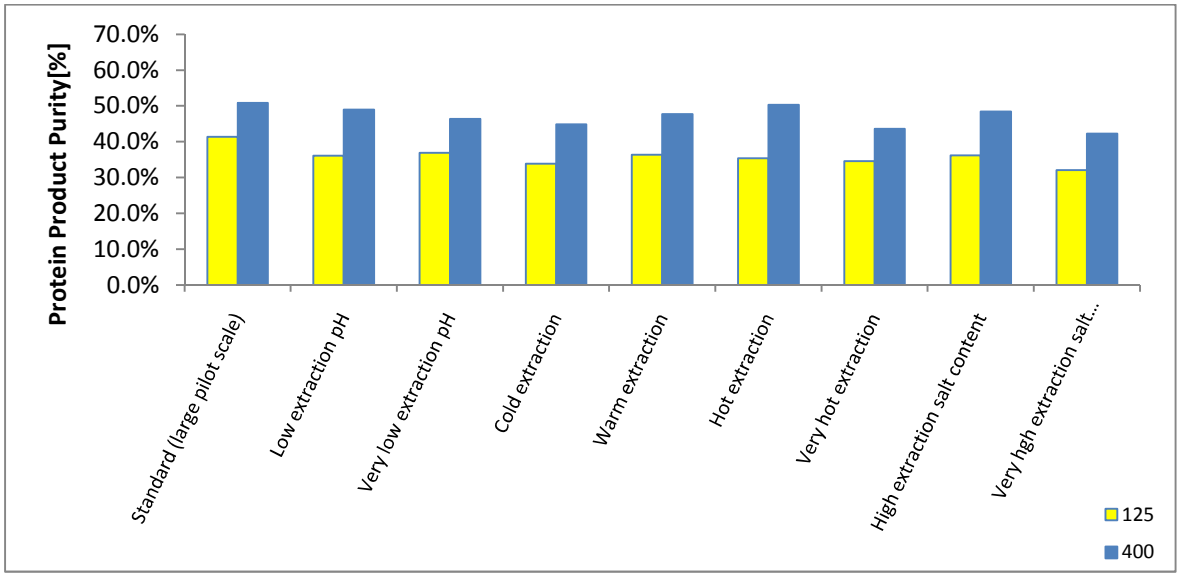


Figure 9.8. Oat-gluten protein purity of samples collected on the 400 µm sieve and the 125 µm sieve for samples with varying extraction conditions.

Table 9.9. Protein Agglomeration Index of All Samples (by mass)

Sample	Description	Agglomeration index
1	Standard conditions – small pilot scale	0.96
B	Oat dough	0.67
C	Gluten-starch dough	1.00
3	Short dough kneading	0.94
2	Long dough kneading	0.93
12	Very long dough kneading	0.93
4	Low sodium chloride content in dough	0.93
5	Very low sodium chloride content in dough	0.92
7	Low gluten content in dough	0.89
8	High gluten content in dough	0.96
A	Standard conditions – large pilot scale	0.96
22	Extract liquor pH 2.7	0.95
23	Extract liquor pH 2.0	0.96
24	Extract liquor temperature T = 15 °C	0.97
25	Extract liquor temperature T = 30 °C	0.97
26	Extract liquor temperature T = 40 °C	0.97
27	Extract liquor temperature T = 50 °C	0.97
28	1 % sodium chloride content in extract liquor	0.97
29	2 % sodium chloride content in extract liquor	0.96

With two exceptions, the protein agglomeration index did not vary greatly between all seventeen samples (Table 9.9). The protein agglomeration index for the protein product from Sample B was 0.68 and Sample C was 1.000. The protein agglomeration index for the remaining fifteen samples ranged from 0.89 to 0.97.

A direct comparison between the protein yield and purity was carried out for samples collected on both the 400 µm sieve and the 125 µm sieve (Figure 9.9). A linear relationship was observed which was different for each sieve.

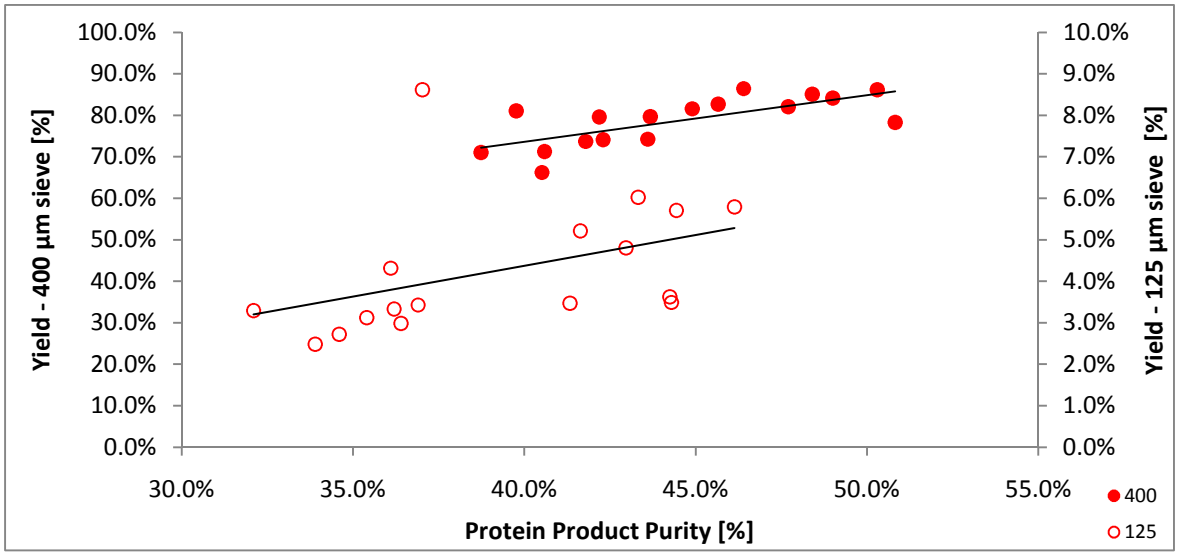


Figure 9.9. Comparison of the purity and yield of the oat-gluten protein product recovered on the 400 µm sieve and the 125 µm sieve.

The relationship (assumed to be linear) between the purity and yield of recovered oat-gluten protein was calculated for the 400 µm sieve and the 125 µm sieves.

- 400 µm sieve

$$YP_{400} = 1.12\,PP_{400} - 0.289 \qquad 19$$

$$R^2 = 0.513$$

- 125 µm sieve including Sample 7

$$YP_{125} = 148\,PP_{125} - 0.016 \qquad 20$$

$$R^2 = 0.174$$

The relationship described by Equation **20** for the 125 µm sieve includes Sample 7 which had a low gluten content in the dough. This sample had a relatively low recovery on the 400 µm sieve coupled with a relatively high recovery on the 125 µm sieve. This resulted in a low protein agglomeration index of 0.86 (Table 9.9). This indicates that there was poor agglomeration of the oat-gluten proteins in this sample, resulting in a poorly formed oat-gluten protein network. It is considered likely that

there was not enough gluten protein present to initiate the agglomeration of the oat-gluten protein network. If Sample 7 is not included in the comparison, the equation the linear relationship between the protein product yield and purity strengthens as shown in Equation 21.

- 125 µm sieve excluding Sample 7

$$YP_{125} = 0.18\,PP_{125} - 0.032 \qquad 21$$

$$R^2 = 0.525$$

9.4.4. Individual Processing Parameter Results

This research investigated the effect on the protein agglomeration as a result of varying oat-gluten dough processing conditions as well as oat-gluten protein extraction conditions. Selected results for individual parameters are presented in the following figures for the yield (Figures 9.10 and 9.15) and purity (Figures 9.16 and 9.21) of the oat-gluten protein.

Protein Yield – Dough Composition

Figure 9.10 shows that there was an optimal oat-gluten dough kneading time for the Al-Hakkak Process. The oat-gluten protein yield initially increased with longer kneading time up to a kneading time of 150 seconds (using the Farinograph mixer) after this the yield decreased. Adding 1 % salt to the dough increased the oat-gluten protein yield initially (Figure 9.11), but further increasing the salt content resulted in a slight decrease in the yield. As shown in Figure 9.12, a positive relationship was observed between the gluten protein content and the oat-gluten protein yield.

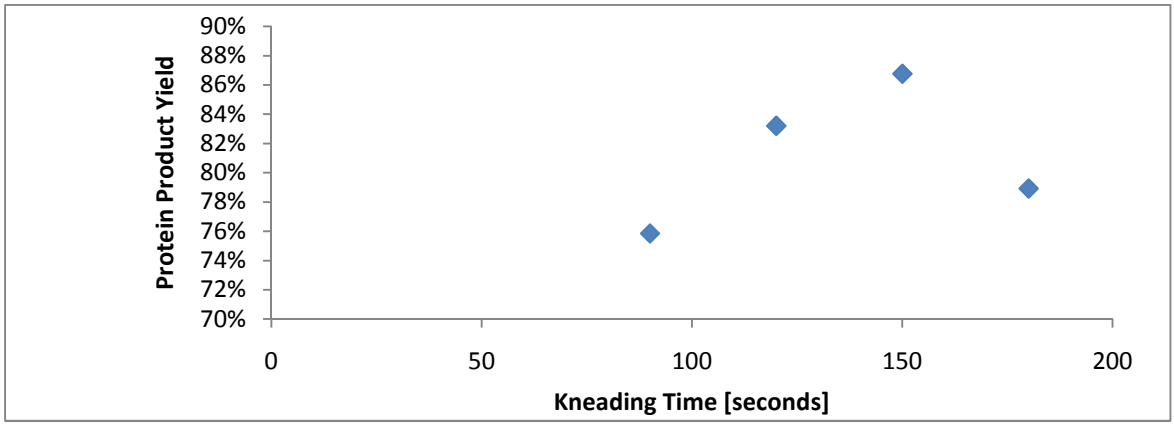


Figure 9.10. The effect of oat-gluten dough kneading time on the total protein product yield.

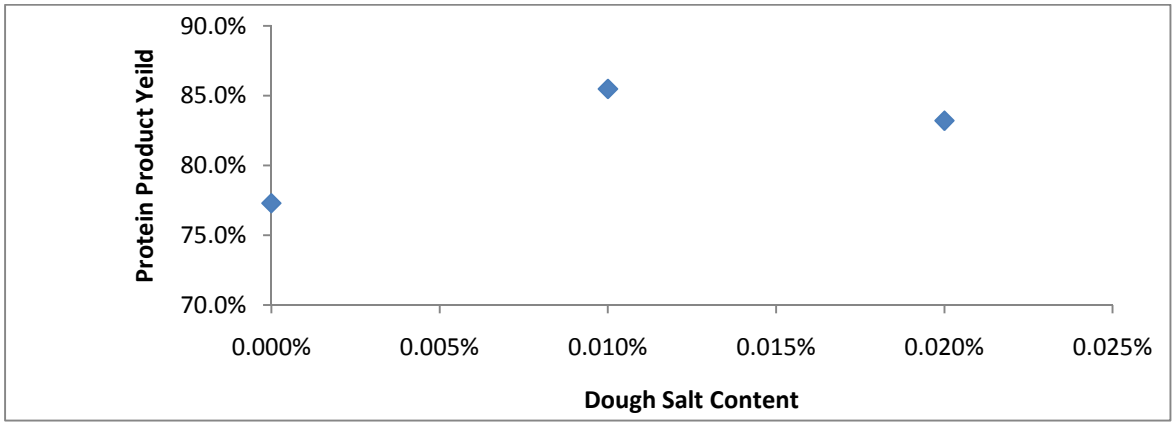


Figure 9.11. The effect of the sodium chloride content of the oat-gluten dough on the total protein product yield.

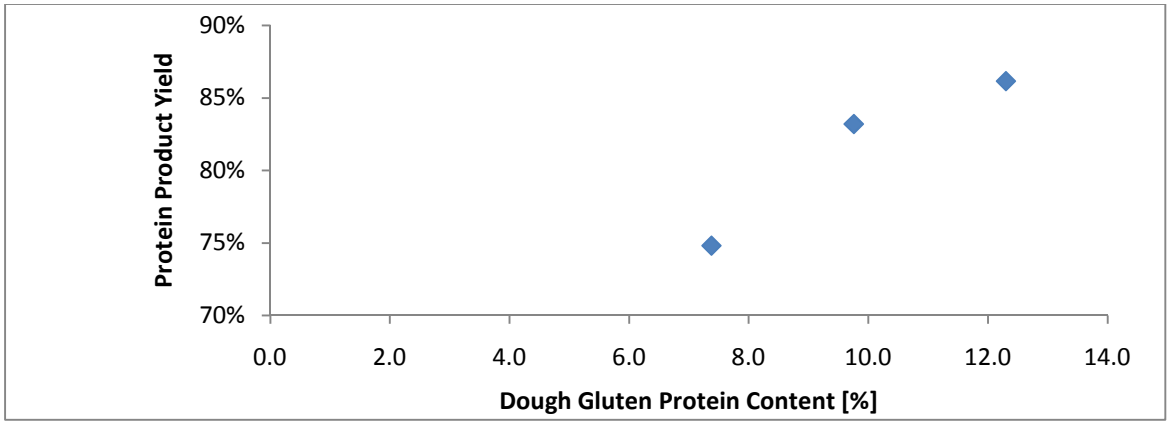


Figure 9.12. The effect of the gluten protein content of the oat-gluten dough on the total protein product yield.

Protein Yield – Extraction

As shown in Figure 9.13 reducing the extract liquor pH increased the protein yield. Figure 9.14 shows that there was an optimal extraction temperature. The oat-gluten protein yield increased with increasing extraction temperature up to 40 °C. But, an extraction temperature of 50 °C resulted in a decrease in yield. An optimal sodium chloride concentration in the extract liquor of 1 % was observed (Figure 9.15) with respect to oat-gluten protein yield.

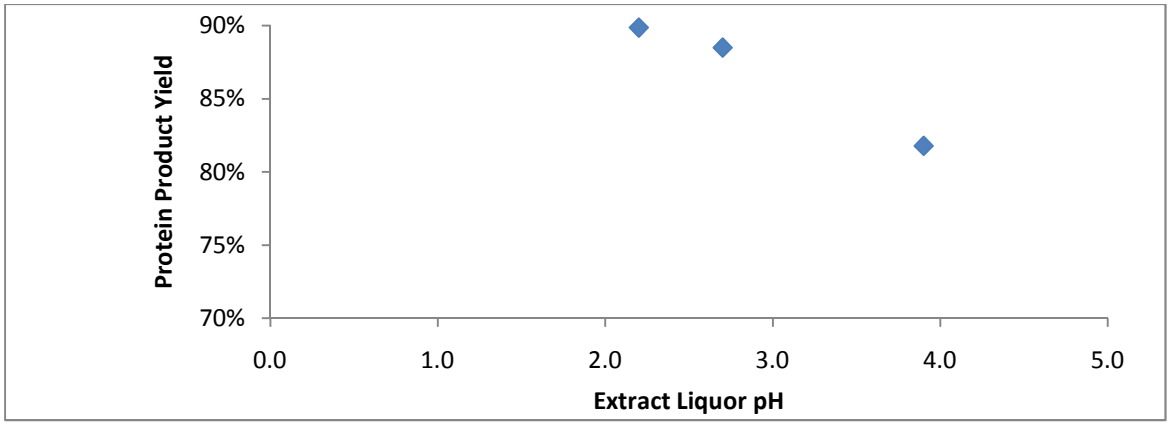


Figure 9.13. The effect of the extract liquor pH on the total protein product yield.

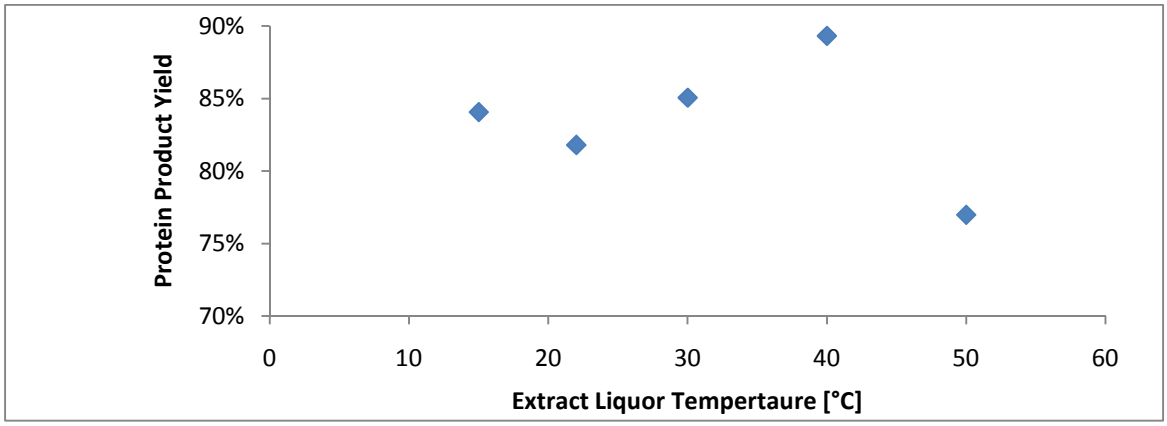


Figure 9.14. The effect of the extract liquor temperature on the total protein product yield.

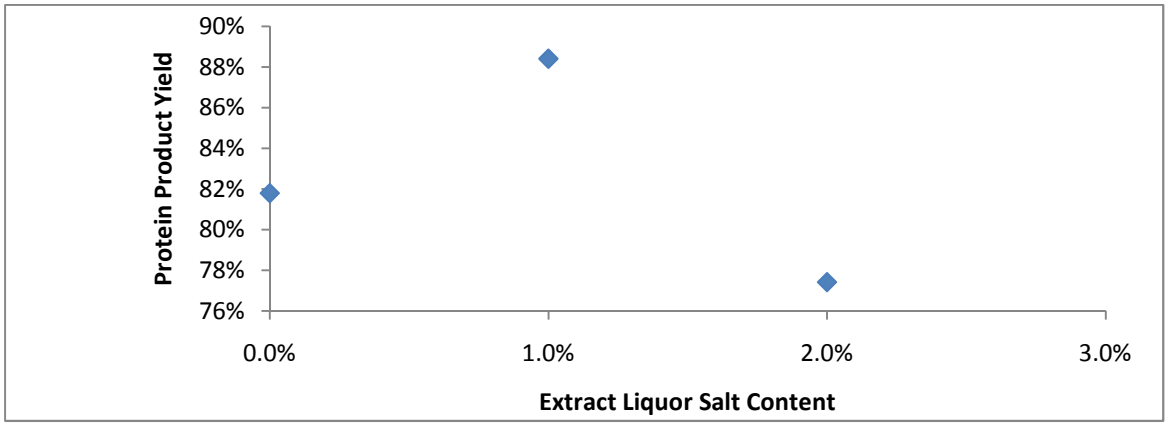


Figure 9.15. The effect of the sodium chloride content of the extract liquor on the total protein product yield.

Protein Purity – Dough Processing

No relationship was observed between kneading time and the oat-gluten protein purity (Figure 9.16). Similarly Figure 9.17 shows no observable influence of the sodium chloride content of the oat-gluten dough on the oat-gluten protein purity. Figure 9.18 shows a positive relationship between the gluten protein content of the dough and the oat-gluten protein purity.

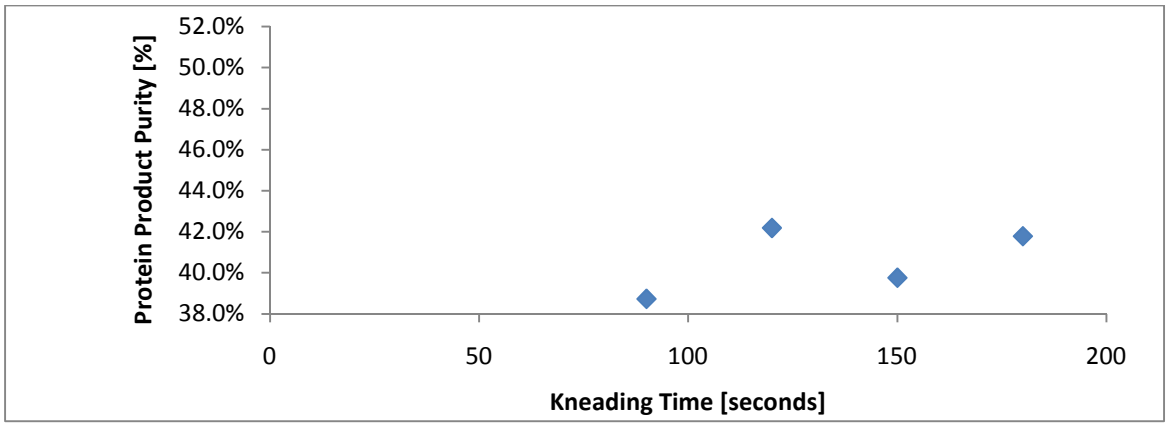


Figure 9.16. The effect of the kneading time of the oat-gluten dough on the purity of the oat-gluten protein product.

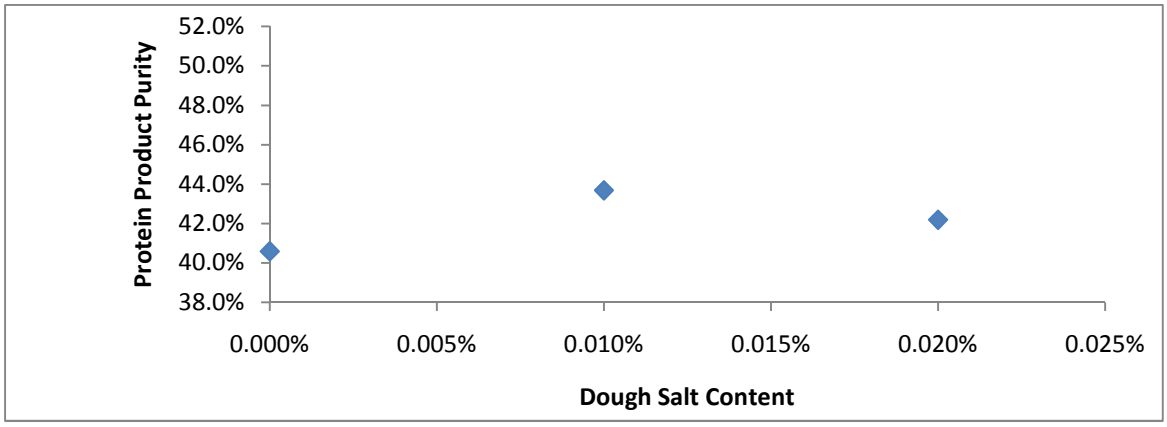


Figure 9.17. The effect of the sodium chloride content of the oat-gluten dough on the purity of the oat-gluten protein product.

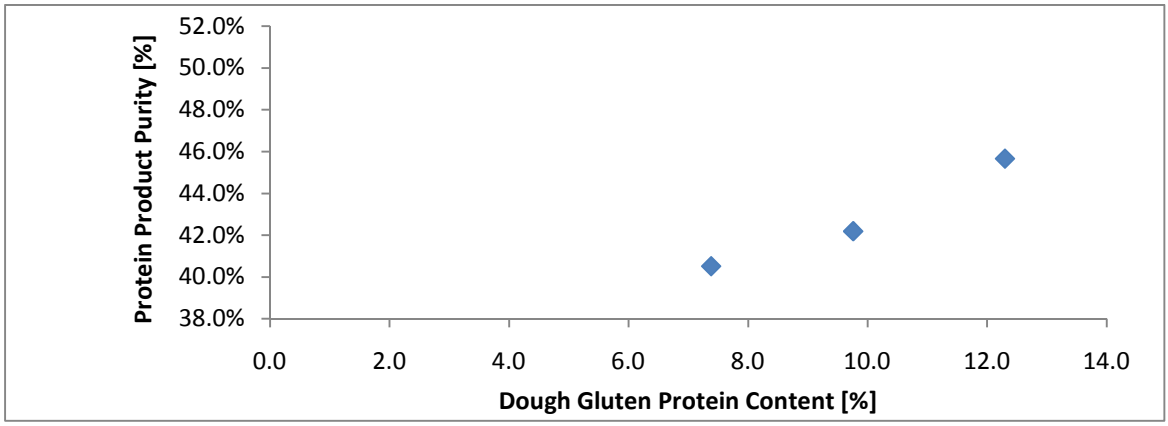


Figure 9.18. The effect of the gluten content of the oat-gluten dough on the purity of the oat-gluten protein product.

Protein Purity – Extraction

Figure 9.19 shows that reducing the extract liquor pH reduced the oat-gluten protein purity, in contrast to Figure 9.13 which shows that a lower pH enhances protein yield. No relationship was observed between the extraction temperature and the oat-gluten protein purity (Figure 9.20). As shown in Figure 9.21, the oat-gluten purity reduced with increasing sodium chloride concentration.

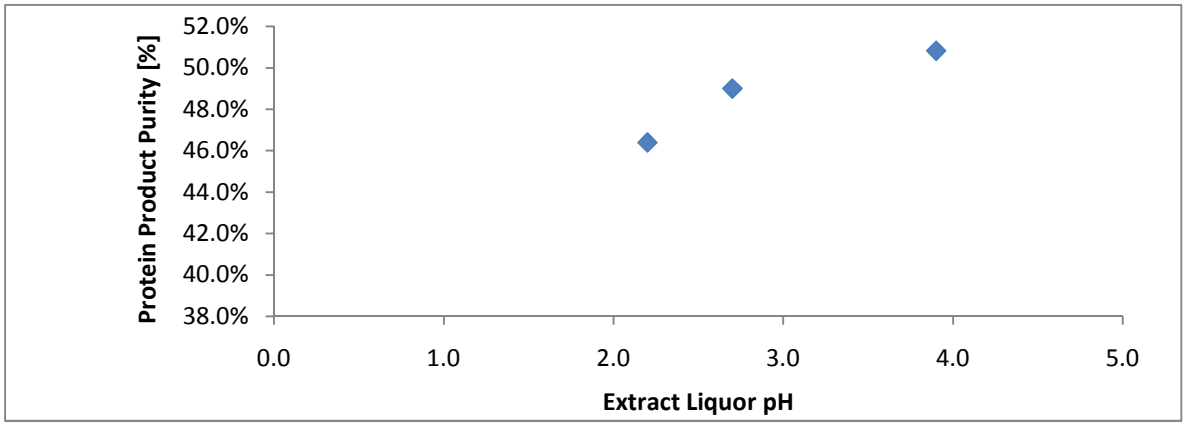


Figure 9.19. The effect of the extract liquor pH on the purity of the oat-gluten protein product.

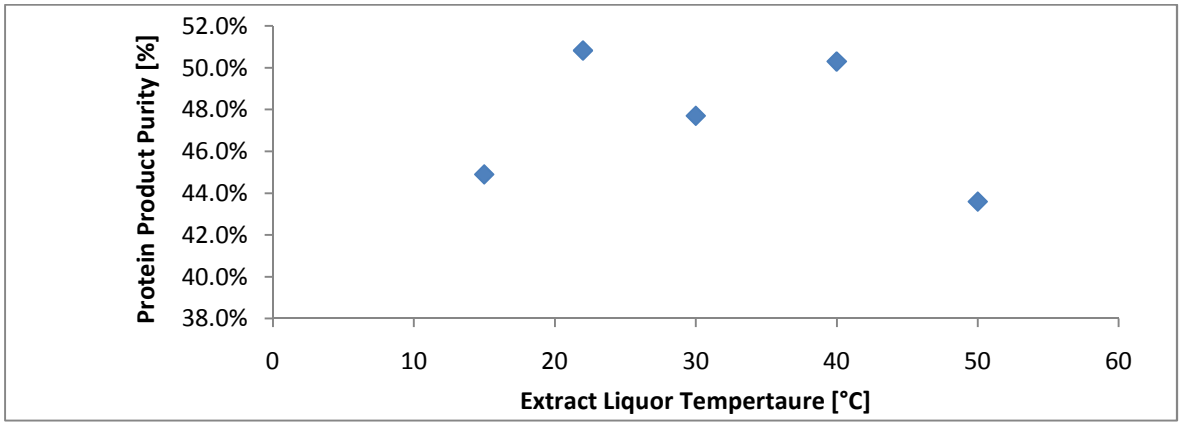


Figure 9.20. The effect of the extract liquor temperature on the purity of the oat-gluten protein product.

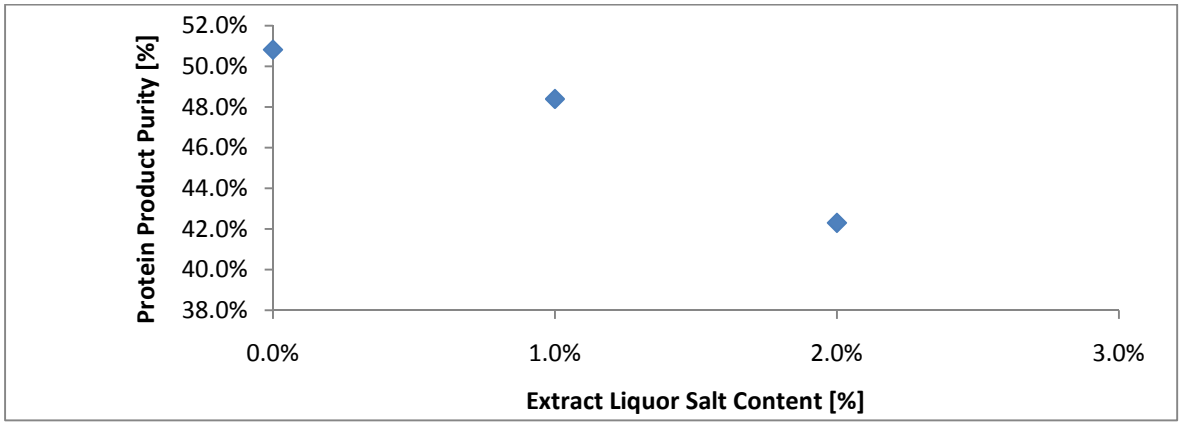


Figure 9.21. The effect of the sodium chloride content of the extract liquor pH on the purity of the oat-gluten protein product.

9.5. Discussion

9.5.1. Oat-Gluten Protein Product Consistency

A qualitative comparison was undertaken between the protein produced by the Al-Hakkak Process using oat-gluten flour (Figure 9.2), oat flour (Figure 9.3), and the gluten-starch flour (Figure 9.4). The gluten-starch protein (Sample C) formed a single, cohesive mass on the 400 µm sieve which had a rubbery consistency. The oat-gluten protein (Sample 1) also formed a single, cohesive mass on the sieve which was pale brown in colour. However, this protein mass was looser (spreading out more over the surface of the sieve) and had a softer consistency. The oat protein (Sample B) did not form a cohesive mass. Instead a slurry formed across the surface of the sieve that could be scraped into a mound using a spatula. This slurry was brown in colour and had a consistency similar to watery porridge.

The appearance of the gluten and oat-gluten protein samples retained by the 400 µm sieve was similar to images presented by Guan et al. [102]. These authors presented images of wheat protein isolated from wheat dough using a process involving wet milling followed by separation and purification using water. They found that wheat dough with a lower gluten protein content resulted in a wheat protein product that was "*slack, spread out more on the sieve, and broke apart into several pieces*". This is consistent with the results from these trials on oat-gluten dough. The oat-gluten flour contained a lower percentage of gluten compared to the gluten-starch flour. The protein from the oat-gluten flour spread out more over the surface of the sieve, although it did not break into several pieces. This qualitatively demonstrates the network-forming functionality of gluten protein component of the oat-gluten dough. This is consistent with the findings from the gluten functionality investigations reported in Chapter 8.

9.5.2. Oat-Gluten Protein Agglomeration Index

With two exceptions, the protein agglomeration index did not vary greatly between all seventeen samples (Table 9.9). The protein agglomeration index for the remaining fifteen samples ranged from 0.885 to 0.970.

- The protein agglomeration index for the protein from Sample B was 0.677. This sample was produced from oat flour without any added gluten. With no gluten protein present in the dough it is apparent that the oat protein did not agglomerate and a protein network did not form. Hence, a low protein agglomeration index was expected.
- Sample C was produced from gluten-starch flour with no oat flour and resulted in an agglomeration index of 1.000. As the only proteins present in this sample were the wheat gluten proteins (with inherently high protein network-forming functionality), a high agglomeration index was expected. In addition, this "manufactured" flour was free from other biopolymer fractions naturally present in flour that could hinder the formation of the protein network, such as soluble sugars, carbohydrates, and lipids [25, 59, 61].

Processing Scale

The mixing action of the small and large pilot scale processing equipment is different. The Farinograph is a two arm, z-style mixer with contra-rotating mixer arms. This style of mixer involves a combination of bulk movement, shear, stretching, folding, dividing, and recombining between the impellor blades and vessel walls [207, 208]. The Hobart is a pan mixer with a rotating hook style impellor. This rotating action creates a scraping action between the impellor and the vessel walls, with the vessel walls providing a stationary surface. Shearing and stretching occur in a localised area between the impellor and the vessel walls. This style of mixer imparts less deformation of the dough during a mixer single rotation compared with the two arm, z-style mixer. Previous investigations had identified that, for the Al-Hakkak Process, the optimal kneading time using the Farinograph was considerably shorter (approximately

2 minutes) than for the Hobart (approximately 10 minutes) [51, 52]. This is most likely due to differences in both the mixing action and the scale of processing.

Table 9.9 shows that both the standard samples produced using small (Sample 1) and large (Sample A) pilot scale mixers and had similar agglomeration index results (0.956 and 0.958 respectively). This indicated that the style of mixer did not influence the protein agglomeration. This is important information for commercial manufacturing as it showed that the style or scale of mixer selected for kneading the dough is not a key factor for the Al-Hakkak Process. These results confirm the validity of using the small pilot scale Farinograph mixer (50 g) to predict the performance of the Al-Hakkak Process using larger scale processing equipment such as the Hobart mixer (5 kg). However, the results have shown that specific processing conditions (such as mixing time) vary considerably for each mixer and must be optimised.

Gluten Content

Table 9.9 shows that reducing the gluten content of the oat-gluten dough by 25 % resulted in a lower the protein agglomeration index (Sample 7 = 0.885) compared to the standard (Sample 1 = 0.956). However, increasing the gluten content by 25 % had very little effect on the protein agglomeration index (Sample 8 = 0.959). This indicates that there was an optimal value for the gluten content of the dough used in the Al-Hakkak Process. Below this, the protein agglomeration reduced, but above it there were minimal gains. This is consistent with observations and conclusions made elsewhere in the thesis. The rheology investigations undertaken as part of this study (discussed in Chapter 5) found that the gluten content influenced the large deformation rheology of sheeted oat-gluten dough. Increasing or decreasing the gluten content by 25 % resulted in a decrease in the apparent modulus of elasticity, strength index and strain hardening index. A conclusion of the rheology trials was that the optimal gluten content was similar to the current standard gluten content of the oat-gluten dough. Chapter 8 of this thesis reported research carried out investigating replacing gluten protein in the Al-Hakkak Process with oat-gluten protein from a previous Al-Hakkak Process run. It was concluded that reducing the total gluten content of the oat-gluten dough reduced both the yield and purity of the oat-gluten protein. A gluten content of

at least 11.3 % was needed for good protein agglomeration and a gluten content of at least 16.1 % for excellent protein agglomeration.

Other studies have reported a relationship between the protein agglomeration index and the gluten content of wheat dough [25, 59, 95, 102]. These studies used flour from different cultivars of wheat with different protein contents. Different cultivars have different compositions (such as soluble proteins, sugars, lipids and carbohydrates) and this complicated the interpretation of the results. These previous studies have also focused on a Batter Process not the Martin Process or a Dough-Batter Process. However, as all of these processes rely on the agglomeration of the gluten proteins the results are considered relevant to this study. Generally the greater the gluten content, the greater the protein agglomeration index for similar types of flour. The trials undertaken for this study on oat-gluten flour used only a single source of gluten flour and a single source of oat flour. The gluten content was varied by changing the amount of added gluten flour which simplified the interpretation of the results.

9.5.3. Oat-Gluten Protein Product Yield and Purity

Overall Effect

Figure 9.9 shows that generally as the oat-gluten protein yield from the Al-Hakkak Process increased (as a percentage of the initial protein content) the purity of the extracted oat-gluten protein increased. This applied to both the oat-gluten protein retained by the 400 µm sieve and the 125 µm sieve. The relationship appears linear. Hence, an optimum oat-gluten protein yield or purity target was not identified, which is important information for commercial manufacturing. A linear relationship has been previously reported between gluten protein recovery and purity for wheat starch and protein separation using a batter process [59]. These authors also found that a flour with a higher gluten protein recovery and purity required longer kneading time when used for bread making (a dough process). This is constant with other observations and conclusions made in this thesis. This observation requires further study.

Chapter 8 of this thesis investigated replacing gluten protein in the Al-Hakkak Process with oat-gluten protein from a previous Al-Hakkak Process run. A similar linear

relationship between oat-gluten protein yield and purity was observed in these gluten reuse trials. However, the relationship for oat-gluten protein reuse was more strongly correlated for the second generation oat-gluten protein product retained by the 400 µm sieve ($R^2 = 0.855$) compared to the results from these trials ($R^2 = 0.513$). This suggests that the relationship between oat-gluten protein yield and purity in the Al-Hakkak Process is complex. In Chapter 8 only a single variable was considered (reusing the oat-gluten protein) and the results generated a strong correlation. These trials (Chapter 9) considered several processing variables. The results suggest that the various processing parameters influence to different magnitudes the relationship between the oat-gluten protein yield and purity. Hence, it can be concluded that generally there was a positive relationship between protein yield and purity, but that the various processing parameters influenced this relationship to different extents.

Kneading Time

The kneading time influenced the oat-gluten protein yield from the Al-Hakkak Process. Figure 9.10 shows that the maximum protein yield (86.8 %) occurred with the sample that was kneaded for 150 seconds (Sample 2) using the Farinograph. Increasing the kneading time to 180 seconds (Sample 12) reduced the yield to 79 % and reducing the kneading time to 90 second (Sample 3) reduced the yield to 76 %. This clearly demonstrates that there is an optimal kneading time for the Al-Hakkak Process.

These results support observations and conclusions made elsewhere in the thesis. The rheology investigations undertaken as part of this study (reported in Chapter 5) found that the kneading time influenced the large deformation rheology of sheeted oat-gluten dough. Very long (180 seconds) and short (90 seconds) kneading times produced weaker dough (lower stress for any given strain). Very long and short kneading times also resulted in a decrease in the apparent modulus of elasticity, strength index, and strain hardening index of the sheeted oat-gluten dough. These rheology trials indentified that the optimum kneading time was between 120 seconds and 150 seconds. Other investigations undertaken reported in this thesis have shown that the structure of the oat-gluten protein network changed with kneading time

(Chapter 6). Confocal scanning laser microscopy techniques were used to visually assess the structure of the oat-gluten protein product from the Al-Hakkak Process. This qualitative assessment technique showed that increasing the kneading time from 90 seconds to 150 seconds resulted in a finer protein network structure. However, confocal scanning laser microscopy did not quantitatively identify whether an optimal kneading time existed.

Wheat dough has been shown to have an optimum kneading time in baking studies [81, 82, 178, 180]. Using an optimal kneading time results in a strong, smooth wheat dough with visco-elastic properties. Both under- and over-kneading produce a weak and sticky wheat dough. A study by Frederix et al. [64] showed a positive relationship between increased kneading time and increased gluten yield using wheat dough in a Batter Process. These authors concluded that gluten protein development as a result of dough kneading was an important factor in the separation of wheat starch and protein. This is consistent with the results from this study on the effect of the kneading time of oat-gluten dough during the Al-Hakkak Process.

Dough Sodium Chloride Content

Adding 0.01 % sodium chloride (salt) by mass to the dough initially increased the oat-gluten protein yield from the Al-Hakkak Process from 77 % to 86 % (Figure 9.15). However, further increasing the dough sodium chloride content to 0.02 % (standard composition) resulted in a slight decrease to 83 %. Increasing the sodium chloride content of the dough had little effect on the protein purity. Hence, improvements were made to the yield by including up to 0.01 % sodium chloride in the dough recipe. Further increasing to the sodium chloride content showed no benefit yield and may have had a slight negative effect. No relationship was observed between dough sodium chloride content and protein purity (Figure 9.17).

Other investigations undertaken as part of this study also indentified a relationship between dough rheology and sodium chloride content (Chapter 5). These results showed that sodium chloride content influenced the large deformation rheology of the oat-gluten dough. Increasing sodium chloride content from 0.0 % to 0.01 % and then

0.02 % progressively strengthened the dough (increased stress for any given strain) and increased the apparent modulus of elasticity, strength index and strain hardening index.

The role of sodium chloride in wheat dough formation was reviewed by Miller and Hoseney [209]. However, the focus of their review was baking and the effect of sodium chloride on starch and protein separation processes was not discussed. The review identified several studies that show changes in the rheology of wheat dough and the water content of the gluten protein product as a result of adding sodium chloride to the dough. The studies reviewed did not relate the results to the separation of protein and starch. The review also identified that it is generally accepted that including sodium chloride in wheat dough recipes reduces the size of the electric field surrounding the positively charged gluten protein molecules, thus allowing the individual molecules to approach each other. The authors concluded that salt facilitated interactions between the protein molecules.

Another study using wheat found that the sodium chloride content of wheat dough improved both the gluten protein recovery and purity in a process involving wet milling followed by gluten and starch separation and purification using water [102]. Van der Zalm et al. investigated the separation of wheat starch and protein using a novel shear-induced separation process [210]. Their research showed that separation was enhanced by the addition of some sodium chloride, but that very high concentrations of sodium chloride did not promote separation. But, no correlation was reported between the small deformation rheology of wheat dough and sodium chloride concentration. These published results are consistent with the outcomes of research presented in this thesis on the influence of the sodium chloride content of oat-gluten dough on the separation of protein and starch during the Al-Hakkak Process.

The influence of sodium chloride on the molecular interaction of gluten proteins was studied by Ukai et al. [211]. The authors concluded that both interactions between proteins and the distances between individual protein molecules were altered by the addition of sodium chloride. The authors showed that the presence of sodium chloride in the dough increased the solubility of the gluten proteins. This would be undesirable for starch and protein separation as it would reduce the insoluble protein product yield.

Increased solubility may have contributed to the reduction in the oat-gluten protein yield observed in this study at the higher sodium chloride content (0.02 %).

It is proposed that including sodium chloride in the oat-gluten dough facilitated interactions between the protein molecules. The size of the electric field surrounding the positively charged oat and gluten protein molecules was reduced and as a result the individual protein molecules were able to approach each other. This enhanced the formation of the oat-gluten protein network which, in turn, facilitated the separation of the insoluble protein network from the starch granule slurry. Thus, the oat-gluten protein yield improved.

Extract Liquor Sodium Chloride Content

Increasing the sodium chloride concentration of the extract liquor from 0 % to 1.0 % sodium chloride (by mass) initially increased the oat-gluten protein yield from the Al-Hakkak Process from 82 % to 88 % (Figure 9.15). However, further increasing the sodium chloride concentration to 2.0 % resulted in a decrease to 77 %. The oat-gluten purity reduced from 50.8 % to 42.3 % at a sodium chloride concentration 0.0 % and 2.0 % respectively in the extract liquor (Figure 9.21). Hence, improvements were made to the protein yield by including up to 1.0 % sodium chloride in the extract liquor. But this gain in oat-gluten protein yield corresponded to a reduction in purity. This trend is similar to the trend observed for the sodium chloride content of the oat-gluten dough, where an optimal concentration was also observed.

No studies have been identified on the sodium chloride concentration of the extract liquor in wheat starch and gluten separation. The results of these trials suggest that interactions between proteins between individual protein molecules were altered by the addition of sodium chloride to the extract liquor.

Similar the effect of sodium chloride in oat gluten dough, it is proposed that including sodium chloride in the extract liquor facilitated interactions between the oat and gluten protein molecules. The size of the electric field surrounding the positively charged oat and gluten protein molecules was reduced during the extraction process and as a result the individual protein molecules were able to approach each other and interact. As

discussed previously, it is likely that increased gluten protein solubility contributed to the reduction in the oat-gluten protein yield at 2 % sodium chloride content.

Dough Gluten Protein Content

A positive relationship was observed between the gluten protein content of the oat-gluten dough and protein yield (Figure 9.12) and purity (Figure 9.18). Increasing the gluten protein content by 25 % increased the oat-gluten protein yield from 83 % to 86 %. This translates to a 14 % increase in the protein yield compared with an increase of 10 % of total protein in the initial dough (oat protein plus gluten protein in the dough). Decreasing the gluten protein content reduced the oat-gluten protein yield to 75 %. This translates to a 21 % decrease in the oat-gluten protein yield compared with a decrease of 12 % of total protein in the initial dough. Hence, more oat-gluten protein was recovered from the Al-Hakkak Process than gluten protein added. However, the magnitude of the improvements diminished as the gluten protein content increased. Similar increase in the oat-gluten protein purity was observed from 42 % to 46 % with the 25 % increase in the gluten protein content and a decrease to 41 % was observed with a 25 % decrease in the gluten protein content. This clearly shows that the gluten protein component of the oat-gluten dough influences the formation of the oat-gluten protein network. Thus, it was concluded that there was an optimal gluten protein content for oat-gluten dough in the Al-Hakkak Process.

The large deformation rheology investigations reported in Chapter 5 support the conclusion that there was an optimal gluten protein content for oat-gluten dough in the Al-Hakkak Process. Changing the gluten content altered the large deformation rheology of the oat-gluten dough. Increasing or decreasing the gluten content by 25 % resulted in a decrease in the apparent modulus of elasticity, strength index and strain hardening index. A conclusion of the rheology trials was that there was an optimal gluten content which was similar to the standard gluten content. Chapter 8 of this thesis describes and discusses investigations carried out into replacing gluten protein in the Al-Hakkak Process with oat-gluten protein from a previous Al-Hakkak Process run. It was concluded from these trials that reducing the total gluten content of the oat-gluten dough reduced both the yield and purity of the protein product. This is

consistent with the results of the investigations reported in this chapter. Hence, a key conclusion of this thesis is that there is an optimal gluten content for oat-gluten dough in the Al-Hakkak Process and that this concentration is similar to the standard gluten concentration. Establishing the optimal gluten protein content for commercial production is expected to include economic considerations.

The effect of gluten protein content on wheat dough rheology and baking is well studied [64, 77, 156, 177, 183]. The studies show that a low gluten content typically produces weak wheat dough whilst higher gluten content typically produces strong wheat dough. A few studies have been undertaken on the effect of gluten protein content of wheat dough on the starch and protein separation [25, 59, 95, 102]. These studies have predominantly focused on the Batter Process and not the Martin Process or Dough-Batter Process. Generally the greater the gluten content, the greater the protein product yield. This is consistent with the results reported in this thesis which have concluded that there is an optimal gluten content for oat-gluten dough in the Al-Hakkak Process.

Extract Liquor pH

Figure 9.13 shows that reducing the extract liquor pH improved the oat-gluten protein yield from 82 % at pH 3.9 (the natural and uncontrolled pH of the extract liquor) to 90 % at pH 2.2. However, there was a corresponding negative effect on the oat-gluten protein purity which reduced from 45.7 % to 40.5 % (Figure 9.19). This means that for commercial production there will always be a trade off between oat-gluten protein yield and purity in the Al-Hakkak Process when varying the pH of the extract liquor.

Some cereal proteins such as the glutenlin fraction are soluble in dilute acid solutions [6, 14, 40, 41]. These proteins are involved in gluten protein agglomeration and the formation of the gluten protein network in wheat dough. Despite this solubility, the oat-gluten protein yield increased when the pH of the extract liquor was reduced in the Al-Hakkak Process. This indicates that lowering the pH of the extract liquor promoted other protein interactions that dominated the effect of any increase in the solubility of the glutenlin proteins.

No studies have been identified that report on the effect of acid addition and reduced pH on the extraction liquor used in wheat starch protein separation processes such as the Martin Process or the Batter Process. A few studies have investigated the effect of ascorbic acid (a common dough additive) on wet separation of wheat starch and protein. All of the studies that have considered ascorbic acid addition have only investigated adding the acid during dough making. Dik et al. [65] found that the addition of low concentrations of ascorbic acid to the flour during dough making (up to 0.5 g/kg) did not alter the aqueous wet separation behaviour for wheat starch and protein using a wet sieving technique. Larsson and Eliasson [212] reported that lowering the pH of wheat dough using low concentrations of ascorbic acid (0.23 g/kg) resulted in an improvement in wheat starch and protein separation using ultracentrifugation separation techniques.

Extract Liquor Temperature

Increasing the temperature of the extract liquor from 22 °C to 40 °C increased the oat-gluten protein yield from 82 % to 89 % as shown in Figure 9.14. However, further increasing the extract liquor temperature to 50 °C resulted in a decrease in the protein yield to 77 %. This shows that a 9 % improvement can be made in the protein yield from the Al-Hakkak Process by increasing the extract liquor temperature to 40 °C. This is important information for a commercial manufacturing process. Operating the Al-Hakkak Process at a high temperature would provide gains in the oat-gluten protein yield, but would generate penalties through increased heating costs. No relationship was observed between extract liquor temperature and oat-gluten protein purity (Figure 9.20).

These results are consistent with other studies on wheat starch and protein separation. Several studies have reported that wheat starch and protein separation improves with increasing temperature. Yondem-Makascioglu et al. [68] found that 40 °C was the optimal extract liquor temperature for wheat starch protein separation using wet sieving in a Dough-Batter Process. These authors found that extraction at 50 °C did not work well with the gluten showing poor agglomeration which hindered sieving. Van der Zalm et al. [210] recently investigated the separation of wheat starch and

protein using a unique shear-induced separation process. These authors showed that separation was enhanced by increasing the temperature to 40 °C, but extraction at 60 °C was not successful. Higher temperatures are reported to favour contacts and reactions between the proteins [25]. However, very high temperatures can denature the proteins. These studies on wheat dough separation are consistent with the outcomes of this study on the influence of temperature on the oat-gluten dough separation in the Al-Hakkak Process.

The onset of gelatinisation of oat starch is reported to occur between 44.7 °C to 47.3 °C [23]. Operating the Al-Hakkak Process using oat-gluten flour above this temperature would cause the starch to begin to gelatinise, altering the solubility and causing the starch granules to swell. This would be undesirable. The increased starch solubility could interfere with the separation of the starch and protein fractions resulting in poor oat-gluten protein yield and purity.

It is concluded that higher extract liquor temperatures, up to a maximum of approximately 40 °C, favoured contacts and reactions between the oat and gluten proteins. This promoted the formation of the protein network and facilitated the separation of the protein and starch granules. This resulted in an improved oat-gluten protein yield. Higher temperatures (>40 °C) damaged the proteins and reduced their protein network-forming functionality. This damage would include denaturisation of the gliadin protein fraction. Raising the temperate above 44.7 °C caused the starch granules to start to gelatinise. This increased the starch solubility which interfered with the oat-gluten protein network and negatively affected the oat-gluten protein yield.

9.6.Conclusions

The hypothesis for these trials was confirmed. Varying the initial dough composition and processing parameters did affect the formation of the oat-gluten protein network, and this influenced the separation of starch and protein during the Al-Hakkak Process. However, not all processing conditions were found to have an optimum operating

range. Varying some processing parameters resulted in a trade off between improved oat-gluten protein yield and purity (for example extract liquor pH). Thus, it was not possible to identify optimal operating conditions for all of the processing parameters.

A positive relationship was identified between increasing oat-gluten protein yield and purity in the Al-Hakkak Process. However, this relationship was found to be complex with the various processing parameters having different magnitude of influence on the oat-gluten protein yield and purity. Further research is required to characterise this relationship.

This study investigated two styles and scales of kneading and extraction equipment: 1) a small 50g, two arm, z-style mixer (the Farinograph), and 2) a larger 5kg, pan mixer with a rotating hook style impellor (the Hobart). Both mixers produced similar protein agglomeration results when appropriate kneading times were used. This confirmed the validity of using the small scale Farinograph mixer to predict the performance of the Al-Hakkak Process using larger scale processing equipment such as the Hobart mixer. This is an important and key discovery for commercial manufacturing. It demonstrates that a small pilot scale dough kneader can be used to investigate changes to a larger manufacturing process as long as the optimal kneading conditions are established for the particular kneader. It also demonstrates that different styles of dough kneader can be used in the Al-Hakkak Process, with equipment selection based on other factors such as availability and cost.

The kneading time was found to have influenced the oat-gluten protein yield from the Al-Hakkak Process with an optimum kneading time established of about 150 seconds (using the Farinograph). This is consistent with observations and conclusions in the rheology investigations undertaken as part of this study (Chapter 5). These rheology investigations concluded that the a kneading time of between 120 second to 150 seconds gave the greatest apparent modulus of elasticity, while shorter and longer kneading times reduced the apparent modulus of elasticity.

It was established that the gluten content of the oat-gluten dough was a key parameter in the Al-Hakkak Process and that the gluten protein component of the oat-gluten dough influences the formation of the oat-gluten protein network. A low gluten

content was shown to reduce the protein agglomeration index indicating that the protein network was poorly formed in the oat-gluten dough. It was concluded that a gluten content of at least 11.3 % was needed for good protein agglomeration and a gluten content of at least 16.1 % for excellent protein agglomeration. Increasing the gluten protein content increased both the oat-gluten protein purity and yield from the Al-Hakkak Process. However, the magnitude of the improvements diminished as the gluten protein content increased. It was concluded that there was an optimal gluten protein content for oat-gluten dough in the Al-Hakkak Process and that this is similar to the standard gluten concentration. Establishing the optimal gluten protein content for commercial production is expected to include economic considerations. These conclusions, regarding the gluten content of the oat-gluten dough, are based on the research outcomes from the protein product yield and purity (this chapter), protein agglomeration index (this chapter), gluten functionality (Chapter 8), and large deformation dough rheology (Chapter 5).

Improvements can be made to the oat-gluten protein yield by including up to 0.01 % sodium chloride in the dough or up to 1 % sodium chloride in the extract liquor. It was concluded that including sodium chloride in the extract liquor facilitated interactions between the oat and gluten protein molecules. The size of the electric field surrounding the positively charged oat and gluten protein molecules was reduced during the extraction process and as a result the individual protein molecules were able to approach each other and interact. It is likely that increased gluten protein solubility contributed to the reduction in the oat-gluten protein yield at 2 % sodium chloride content. The large deformation rheology investigations undertaken as part of this study (reported in Chapter 5) were consistent with this. These rheology trials showed that reducing the oat-gluten dough sodium chloride concentration resulted in a reduction in the apparent modulus of elasticity.

The results of these investigations have shown that reducing the pH during the extraction step improved the oat-gluten protein yield and also reduced the oat-gluten protein purity. Hence, it was concluded that there was a trade off between protein yield and purity in the Al-Hakkak Process. It was concluded that the lower pH of the

extract liquor promoted protein interactions and that these protein interactions dominated any effect of increased protein solubility due the acidic conditions.

Improvements were made in the oat-gluten protein product yield from the Al-Hakkak Process by increasing the extract liquor temperature to 40 °C whist increasing the temperature further to 50 °C had a negative impact. It is proposed that higher extract liquor temperatures, up to a maximum of approximately 40 °C, favoured contacts and reactions between the oat and gluten proteins. This promoted the formation of the protein network and facilitated the separation of the protein and starch granules. This resulted in an improved oat-gluten protein yield. Higher temperatures (>40 °C) damaged the proteins and reduced their network-forming functionality. This damage would include denaturisation of the gliadin protein fraction. Raising the temperate above 44.7 °C caused the starch granules to start to gelatinise. This increased the starch solubility which interfered with the oat-gluten protein network and negatively affected the oat-gluten protein yield.

10. General Discussion and Summary

The proposed overall purpose of this research project was to investigate the Al-Hakkak Process as a method of separation of various biopolymer fractions from cereals that contain little or no gluten. An important factor for success was to separate these biopolymer fractions with minimal alteration to the chemical and morphological structure of the individual molecules (for example, the physical confirmation of the protein molecules). In this way the inherent natural functionality and characteristics of these naturally occurring biopolymers would be maintained.

Oat was selected as the candidate cereal as it does not contain wheat-like gluten proteins, is low value, readily available in New Zealand as well as globally, and contains biopolymers with interesting natural functionality such as starch with a small granule size and proteins rich in the amino acid cysteine. Such functional biopolymers have applications as ingredients in high value, "niche" speciality chemicals products.

The Al-Hakkak Process involves the addition of gluten protein flour from wheat to a non-wheat flour, in this case oat flour. When water is added, the gluten proteins promote agglomeration of the proteins present to form a relatively stable protein network, similar to wheat processing. Prior to this study, research had only been carried out in the laboratory, with many of the processing steps only suited to very small scale, laboratory techniques.

10.1. Challenges

This research project had two distinct, but related challenges; 1) raw material variability, and 2) final product uniformity. Naturally occurring raw materials can vary in composition depending on a range of factors, such as the growing conditions of the plant, geographical location, grain storage conditions and seasonal factors. The development of a robust pilot scale Al-Hakkak Process required gaining an understanding of the impact of these variations on the processes involved. The final product must be consistent and uniform and be in the desired form for commercial

sale. The Al-Hakkak Process needed to have processing flexibility to manage these natural variations.

10.2. Objectives

The overall objective of this research was to create and test pilot scale processes for the separation of the protein fraction and starch fraction from oat using the Al-Hakkak Process. This has been achieved through a programme of research that builds a robust base of knowledge on the unit operations involved in the Al-Hakkak Process. Several processing steps have been identified and investigated, which have been found to be important to the successful creation of a pilot scale Al-Hakkak Process. The research has examined the oat-gluten dough characteristics, the formation of the protein network in the oat-gluten dough, and the separation of the hybrid oat-gluten protein and starch fractions as well as oat starch drying. This has resulted in interesting, encouraging and some unexpected outcomes which have been discussed in previous chapters of this thesis.

10.2.1.Specific objectives

Specific objectives of the research project were identified in Chapter 1 and these have been achieved. Each specific objective is discussed below.

Investigate the importance of oat-gluten dough rheology on the separation of protein and starch using the Al-Hakkak Process.

The rheology of oat-gluten dough was investigated and characterised using a large deformation technique (Chapter 5). Variable processing parameters were explored, including varying the oat-gluten dough composition and processing conditions.

It was concluded that changes (either chemical or physical) occurred in the oat-gluten protein network of the sheeted oat-gluten dough. Stress, strain, strain hardening, strength index, and apparent modulus of elasticity of sheeted oat-gluten dough were all found to change over time. A characteristic resting time of approximately 14 minutes

was calculated based on the strength index and apparent modulus of elasticity. A key conclusion was that oat-gluten dough resting had an endpoint after which further "resting" was not advantageous. It was proposed that the characteristic resting time could provide a mechanism for quantitatively determining the optimal resting time. A resting time end point is useful for a manufacture process, as it would permit the optimum resting time to be determined and production times optimised.

Kneading time, gluten content, and salt content were identified as key factors influencing oat-gluten dough rheology. Short kneading time or low gluten content produced weak oat-gluten dough. Conversely longer kneading time or higher gluten content produced strong oat-gluten dough. Over-kneading resulted in a weak dough. Thus, it was concluded that there was an optimum operating point for these composition and processing variables.

Another important conclusion was that the changes that took place in the sheeted oat-gluten dough were similar, but not identical, to the changes that occur in sheeted wheat dough. It was proposed that the mechanism for dough development of the sheeted oat-gluten dough differs from sheeted wheat dough for two main reasons; a) the presence of the oat flour disrupts the normal wheat gluten behaviour (for example by diluting the functionality), and b) components in the oat flour alter the activity of the gluten proteins (for example enzymatic cleavage of the gluten proteins).

Understand the structure and functionality of the protein network formed in the Al-Hakkak Process and relate this to the separation efficiency of protein and starch.

Throughout this project the behaviour of the oat proteins and gluten proteins in the oat-gluten dough was the underlying foundation for the research. Gaining an understanding on the formation of the hybrid oat-gluten protein network in the oat-gluten dough and its influence on the separation of starch and protein fractions was considered the crux of the research being undertaken. Three different approaches were

taken. Each provided unique information on the formation and activity of the protein network formed in the oat-gluten dough.

i. Confocal scanning laser microscopy (Chapter 6) provided a visual confirmation of the presence of the protein network in the oat-gluten dough. New and useful knowledge was gained on the overall structure of the protein network and the location of starch granules within that network. There was evidence that changes occurred in the oat-gluten protein network as a result of varying both dough kneading and extraction conditions in the Al-Hakkak Process. The hypothesis was confirmed that both kneading and extraction processes contributed to the formation of a protein network in the oat-gluten dough. Both processes were found to have influenced the structure of the oat-gluten protein network and the location of starch granules trapped within it. However, the effect of kneading time was not as pronounced as the effect of extraction time. A longer kneading or extraction time aligned the protein network into stringy, directional and smooth structure, whereas a shorter kneading or extraction time produced a protein network that appeared gritty with fewer directional strings. Differences in the intensity of the protein staining were observed. It was concluded that the protein molecules were altered during the mixing process and as a result the ability for these molecules to attach to fluorescing molecules during staining was altered.

ii. Gel electrophoresis using reduced and non-reduced conditions provided evidence that the oat and gluten proteins interact at a molecular level in the oat-gluten dough (Chapter 7). This was achieved by comparing the reduced and non-reduced gels of the oat protein and gluten protein with the reduced and non-reduced gels of the oat-gluten protein produced by the Al-Hakkak Process. An important conclusion was that the oat and gluten proteins interacted at a molecular level through reducible, covalent, bonding (most likely disulphide bonding) to form a hybrid oat-gluten protein network. It was proposed that these reducible bonds coupled the individual protein subunits to form new hybrid oat-gluten protein molecules (a combination of oat proteins and gluten proteins). The Al-Hakkak Process provided the necessary conditions (water and energy during mixing) for the protein coupling to occur. Both insoluble

and soluble proteins in the oat and gluten flour were involved in the formation of the hybrid oat-gluten protein network. This new knowledge has implications beyond the Al-Hakkak Process, as it can be applied to a wide range of other dough processing industries, such as the bread, cookie, and pasta making.

iii. The network-forming functionality of the hybrid oat-gluten protein product from the Al-Hakkak Process was established (Chapter 8). It was concluded that the wheat gluten was the source of the protein network-forming functionality of the hybrid oat-gluten protein and that the oat proteins had a diluting effect. Increasing the wheat gluten protein content of the initial oat-gluten dough promoted the formation of the large protein agglomerates that make up the cohesive protein network in the dough. Conversely, the oat protein present in the initial dough had a deleterious effect on the functionality of the hybrid oat-gluten protein. The source of the gluten proteins did not influence the functionality (either from gluten flour or from oat-gluten protein flour recycled from a previous Al-Hakkak Process batch). Hence, it was concluded that oat-gluten protein flour from the Al-Hakkak Process could be recycled and used to replace the commercial wheat gluten flour in subsequent production batches. However, due to the diluting effect of the oat proteins, a greater amount of oat-gluten protein would be required to achieve the same degree of protein network formation as gluten flour. Economic considerations would be a factor in any decision to reuse oat-gluten protein in place of gluten protein in the Al-Hakkak Process.

Investigate the effect of variable oat-gluten dough composition on the performance of the Al-Hakkak Process.

The influence of the initial oat-gluten dough composition on the separation of starch and hybrid oat-gluten protein was investigated and established for several parameters (Chapter 9). Parameters that could be readily varied in the oat-gluten dough were selected for investigation, such as kneading time, gluten protein content, and sodium chloride addition (salt). Kneading time of the oat-gluten dough influenced the oat-

gluten protein product yield from the Al-Hakkak Process (Chapter 9) and an optimum kneading time of about 150 seconds was identified using the Farinograph (equivalent to approximately 10 minutes in the Hobart mixer). The gluten protein content was identified as a key parameter in the agglomeration of the proteins in the oat-gluten dough and the formation of the protein network. An optimal gluten content was identified. This was confirmed in the investigations into the oat-gluten protein functionality (Chapter 8). An optimal concentration of sodium chloride in the oat-gluten dough was also determined. It was proposed that this is due to the addition of sodium chloride altering the interactions between individual protein molecules.

These results and conclusions on the influence of the initial oat-gluten dough composition on the separation of starch and protein, are consistent with conclusions made from the rheology investigations (Chapter 5). Thus, it was proposed that large deformation rheology could be a useful tool for predicting the performance of the Al-Hakkak Process.

Investigate the effect of varying processing conditions on the performance of the Al-Hakkak Process.

The influence of operating conditions on the separation of protein and starch was investigated and established for several parameters (Chapter 9). Parameters that could be readily adjusted during commercial manufacturing were selected for this investigation. An optimal concentration of sodium chloride in the extract liquor was identified. It was concluded that this is due to changes in the interactions between individual protein molecules. Reducing the pH during the extraction step improved the oat-gluten protein product yield but reduced the purity. It was concluded that this was due to the lower pH of the extract liquor which promoted protein interactions and that this dominated any effect of increased protein solubility due the dilute acidic conditions. The temperature of the extract liquor was an important parameter in the agglomeration of the proteins in the oat-gluten dough and the formation of the protein network. Increasing the extract liquor temperature to 40 °C increased the protein product yield. Higher temperatures favour contacts and reactions between the proteins

and it was concluded that increasing the temperature promoted interactions between the protein molecules. However, higher temperatures (50 °C) were found to damage the agglomeration and protein network-forming functionality of the gluten proteins.

Drying trials (Chapter 4) established that changes to the processing conditions altered the structure of the spray dried agglomerates of oat starch granules. Soluble biopolymers in the extract liquor acted as an adhesive and glued individual starch granules together to form spherical agglomerates when oat starch slurry was spray dried. Acidification of the extract liquor enhanced this agglomeration. It was proposed the individual starch granules were sticker during spray drying due to the partial acid hydrolysis of the starch granule, which reduced the gelatinisation temperature. Tray drying the oat starch slurry and milling to form a powder product resulted in undesirable damage to the individual starch granules. The hypothesis that drying conditions can be controlled to produce a fine powder of individual, undamaged, oat starch granules was confirmed.

Establish and test the Al-Hakkak Process at pilot scale using commercially available equipment.

The Al-Hakkak Process was successfully tested using pilot scale equipment that was similar to that used in commercial processes. The process was successfully tested using two styles and scales of equipment: 1) a small 50g, two arm, z-style mixer (the Farinograph), and 2) a larger 5kg, pan mixer with a rotating hook style impellor (the Hobart). This is important information for commercial manufacturing, as it shows that equipment selection can be based on other factors such as availability and cost. The validity was confirmed of using the smaller Farinograph mixer to predict the performance of the Al-Hakkak Process using larger scale processing equipment such as the Hobart mixer. Hence, a small scale mixer can be used to investigate changes to a larger scale manufacturing process as long as the optimal kneading conditions are established for the particular kneader being used. This research has confirmed the robustness of the Al-Hakkak Process to both changes in processing scale and equipment.

Key operating parameters have been established for the Al-Hakkak Process using oat-gluten dough. These are summarised in Appendix E.

10.3. Research Approach and Outcomes

The contribution of this research project to the body of knowledge is broad. The research is the first comprehensive and systematic analysis of the Al-Hakkak Process and has provided an understanding of the key fundamental mechanisms underlying the processes. The outcomes of this research project have provided a robust platform for future research and commercialisation of the Al-Hakkak Process.

Large deformation rheology investigations (Chapter 5) provided evidence of the formation of a protein network in oat-gluten dough, similar to the gluten protein matrix that forms in wheat dough. The rheology data showed that the oat-gluten dough had visco-elastic properties (similar, but not identical to the wheat dough) and a key conclusion was that this was due to the formation of an insoluble protein network. The characteristics of this protein network were found to change with changing processing conditions as well as over time. Some key processing parameters were identified for further research.

The formation of an oat-gluten protein network was confirmed in the confocal scanning laser microscopy investigations (Chapter 6). The images show a protein network was present in the oat-gluten dough following the initial starch extraction and separation. The structure of the protein network was shown to change with changing kneading time and/or extraction time. The location of the individual starch granules relative to the protein network was also found to be influenced by the kneading time and/or extraction time.

Gel electrophoresis investigations proved to be a useful technique for investigating the molecular interactions between the proteins in the oat-gluten dough. A key conclusion was that during the Al-Hakkak Process the oat and gluten proteins interacted to form new, hybrid, oat-gluten protein molecules and that these hybrid proteins comprised the

protein network (Chapter 7). Both the soluble and insoluble oat proteins were involved in the interactions with the gluten proteins. The chemical pathway was found to be through reducible inter-molecular bonds, most likely disulphide linkages. It was concluded that the Al-Hakkak Process provided the necessary conditions (water and energy during mixing) for these reducible bonds to form, coupling the oat and gluten protein subunits. This was the crux of the research.

The functionality of the oat-gluten protein network was established in Chapter 8. It was concluded that the gluten proteins provided the underlying protein network-forming functionality. Whilst the oat proteins are involved in the formation of the protein network, their presence diluted the protein network-forming functionality.

In Chapter 9, investigations focused on the impact of various key processing parameters (indentified during the earlier research) on the agglomeration, yield, and purity of the protein produced by the Al-Hakkak Process. Both dough composition and operating parameters were investigated. Processing parameters investigated included gluten content, salt content, and kneading time of the oat-gluten dough as well as pH, salt content and temperature of the extract liquor. An understanding of the overall process was gained through these investigations which provided a robust basis for future commercialisation. A key conclusion was that some processing parameters had an optimal operating condition such as flour particle size, extract liquor temperature, and kneading time. The results were consistent with the large deformation rheology results and it was concluded that this technique was a useful tool for predicting the performance of the Al-Hakkak Process.

Scanning electron microscopy provided valuable information on the agglomeration of spray dried oat starch granules extracted using the Al-Hakkak Process (Chapter 4). It was concluded that the presence of soluble biopolymers from the oat flour caused roughly spherical agglomerates to form when the oat starch slurry was spray dried. These biopolymers acted as an adhesive and glued individual starch granules together to form the agglomerates. It was found that the structure of these agglomerates could be altered by changing the extraction and purification conditions. A mechanism for the formation of the agglomerates was proposed.

10.4. Future Outlook

The research outcomes have provided a robust basis for the commercialisation of the Al-Hakkak Process. However, there remains a great deal to learn about the processes and the interactions between the oat and gluten proteins. Several opportunities for future research have been identified.

a) Oat and Gluten Protein Interactions

A key conclusion from this research was that during the Al-Hakkak Process the oat and gluten proteins interacted forming new, hybrid oat-gluten proteins through reducible, inter-molecular bonds (most likely disulphide linkages). The research proposes that these reducible bonds couple oat and gluten protein subunits to form new hybrid oat-gluten protein molecules. Further investigation is recommended to establish which specific proteins and amino acid groups are involved in the protein coupling. This research would identify the specific inter-molecular bonds that were forming and the structure of the new hybrid oat-gluten protein molecules. Expanding the research to include interactions of gluten with other plant proteins, such as barley amaranth, quinoa, rice, and pea is recommended as this would provide greater insight into the mechanism involved in the interactions. The knowledge gained from this research would go beyond cereal starch and protein separation and could be applied to other dough processes such as baking, bread, and pasta making.

b) Al-Hakkak Process Characterisation

This research concluded that varying the initial dough composition and subsequent dough and extraction processing parameters influenced formation of the oat-gluten protein network. This, in turn, altered the separation of starch and protein during the Al-Hakkak Process. However, there is considerable opportunity to further characterise the process for oat flour to gain more understanding of the relationship between the structure of the protein network and the separation of the starch granules. Further research characterising the Al-Hakkak Process for other sources of flour such as barley, amaranth, quinoa, rice, and pea would provide additional commercialisation options.

c) Other Starch Protein Separation Technologies

Future research applying the hybrid oat-gluten protein to other separation technologies is recommended as these would provide alternative commercialisation options. Other separation processes, such as those listed in Chapter 2, may provide suitable conditions for the for the hybrid oat-gluten protein network to form. Of particular interest is the novel method for mechanically separating wheat starch and gluten which exploits the different rheological properties of the protein network (visco-elastic) and the starch (dilatant) [56, 58, 67, 72]. The key advantage of this method (being developed at Wageningen University in The Netherlands) is the low water requirement compared to traditional separation processes.

d) Starch Granule Spray Drying

This research proposed that the soluble biopolymers provided a mechanism for the individual starch granules to adhere together during and after the spray drying process. Further investigation into the influence of the soluble biopolymers on the drying process is recommended to gain a greater understanding of the influence of the composition of the extract and purification liquors on the final spray dried starch granule agglomerates. Research into the influence of starch granule size is recommended as starch granule agglomerates have only been previously observed for small granule starches (such as amaranth and rice starch) and not for larger granule starches (such as wheat, potato, and barley starch). Further investigation into the mechanism behind the spherical structure of the oat starch granule agglomerates is recommended to establish if this is due to the hydrophobic starch granules migrating towards the outside of the water droplets during spray drying.

11. References

1. Pomeranz, Y., *Industrial uses of oats*, in *The Oat Crop Production and Utilisation*, Welch, R.W., Editor. 1995, Chapman & Hall: London.

2. Haard, N., Odunfa, S., Lee, C., Qunitero-Ramirez, R., Lorence-Quinones, A., and Wacher-Radarte, C., *Fermented cereals. a global perspective*. FAO Agricultural Services Bulletin No 138. 1999, Rome: Food and Agriculture Organisation of the United Nations.

3. Marshall, H. and Sorrells, M., *Oat Science and Technology*. Agronomy, ed. Mickelson, S. Vol. Number 33. 1992, Wisconsin, USA: American Society of Agronomy Inc. Chapter 9, Pages 247 - 263.

4. FAOSTAT, *PriceSTAT*. 2009, Food and Agriculture Organisation of the United Nations.

5. Webster, F., *Oats: Chemistry and Technology*. 1 ed, ed. Webster, F. 1986, Minnesota: American Association of Cereal Chemists Inc.

6. Pomeranz, Y., *Modern Cereal Science and Technology*. 1987, New York: VCH Publishers.

7. Henry, R. and Kettlewell, P., *Cereal Grain Quality*. 1996, London: Chapman & Hall.

8. Gooding, M. and Davies, W., *Wheat Production and Utilization Systems, Quality and the Environment*. 1997, Oxon, UK.: CAB International.

9. Bushuk, W. and Rasper, V.F., *Wheat Production, Properties and Quality*. 1994, London: Chapman & Hall.

10. Welch, R., *The Oat Crop Production and Utilisation*. 1 ed, ed. Welch, R.W. 1995, London: Chapman & Hall.

11. Coffman, F., *Oats and Oat Improvement*. 1 ed. Agronomy, ed. Coffman, F.A. Vol. 8. 1961, Wisconsin: American Association of Agronomy.

12. Webster, F., *Oats*, in *Cereal Grain Quality*, Henry, R.J. and Kettlewell, P.S., Editors. 1996, Chapman & Hall: London.

13. White, E., *Structure and development of oats*, in *The Oat Crop Production and Utilisation*, Welch, R.W., Editor. 1995, Chapman & Hall: London.

14. Welch, R., *The chemical composition of oats*, in *The Oat Crop Production and Utilisation*, Welch, R.W., Editor. 1995, Chapman & Hall: London.

15. Petersen, D., Wesenberg, D., Burrup, D., and Erickson, C., *Relationships among agronomic traits and grain composition in oat genotypes grown in different environments.* Crop Science, 2005. **45**(4): p. 1249-1255.

16. Doehlert, D., McMullen, M., and Hammond, J., *Genotypic and environmental effects of grain yield and quality of oat grown in North Dakota.* Crop Science, 2001. **41**: p. 1066-1072.

17. Youngs, V., Petersen, D., and Brown, C., *Oats*, in *Advances in Cereal Science and Technology*, Webster, F.H., Editor. 1982, American Association of Cerial Chemists Inc.: Minnisota.

18. Western, D. and Graham, W., *Marketing, processing, uses and composition of oats and oat products*, in *Oats and Oat Improvement*, Coffman, F.A., Editor. 1961, The American Society of Agronomy Inc: Wisconsin. p. 552-578.

19. McKechnie, R., *Oat products in bakery foods.* Cereal Foods World, 1983. **28**(10): p. 635-637.

20. MacArthur, L. and D'Appolonia, B., *Comparison of oat and wheat carbohydrates. ii. starch.* Cereal Chemistry, 1979. **56**(5): p. 458-461.

21. Peterson, D., *Composition and Nutritional Characteristics of Oat Grain and Products*, in *Oat Science and Technology*, H G Marshall, M.E.S., Editor. 1992, American Society of Agronomy Inc: Wisconsin, USA. p. Chapter 10, Pages 265 - 292.

22. Paton, D., *Oat starch. part 1, extraction, purification and pasting properties.* die starke, 1977. **29, No 5**(May 1977): p. 149-180.

23. Tester, R. and Karkalas, J., *Swelling and gelatinization of oat starches.* Cereal Chemistry, 1996. **73**(2): p. 271 - 277.

24. Stone, B., *Cereal Grain Carbohydrates*, in *Cereal Grain Quality*, Henry, R. and Kettlewell, P., Editors. 1996, Chapman & Hall.

25. Borght, A.v.d., Goesaert, H., Veraverbeke, W., and Delcour, J., *Fractionation of wheat and wheat flour into starch and gluten: overview of the main processes and the factors involved.* Journal of Cereal Science, 2004. **41**(2005): p. 221-237.

26. Ellis, R., Cochrane, P., Finlay, M., Dale, B., Duffus, C., Lynn, A., Morrison, I., Prentice, D., Swanston, J., and Tiller, S., *Starch Production and Industrial Use.* Journal of Science of Food and Agriculture, 1998. **77**: p. 289-311.

27. Wang, L. and White, P., *Structure and properties of amylose, amylopectin, and intermediate materials of oats.* Cereal Chemistry, 1994. **71**(3): p. 263-268.

28. Wang, L. and White, P., *Structure and physicochemical properties of starches from oats with different lipid contents.* Cereal Chemistry, 1994. **71**(3): p. 443-450.

29. Morrison, W., *Wheat lipids: structure and functionality*, in *Wheat Production, Properties and Quality*, Bushuk, W. and Rasper, V.F., Editors. 1994, Chapman & Hall: London.

30. Sowa, S. and White, P., *Characterisation of starch isolated from oat groats with different amounts of lipid.* Cereal Chemistry, 1992. **69**(5): p. 521-527.

31. Dahlberg, B., *A new process for the industrial production of wheat starch and wheat gluten.* die starke, 1978. **30**(1): p. 8-12.

32. Makela, M. and Laakso, S., *Studies on oat starch with a celloscope: granule size and distribution.* die starke, 1984. **36, No 5**(1984): p. 159-163.

33. Hoover, R. and Vasanthan, T., *Stuies on isolation and characterzation of starch from oat (avena nuda) grains.* Carbohydrate Polymers, 1992. **19**: p. 285-297.

34. Stauffer, C., *Principles of dough formation*, in *Technology of Breadmaking*, Cauvain, S. and Young, L., Editors. 2007, Springer: UK.

35. Wang, L. and White, P., *Functional properties of oat starches and relationships among functional and structural characteristics.* Cereal Chemistry, 1994. **71**(5): p. 451-458.

36. Manthey, F., Hareland, G., and Huseby, D., *Soluble and insoluble dietary fibre content and composition in oat.* Cereal Chemistry, 1999. **76**(3): p. 417-420.

37. Welch, R. *Oat quality - present status and future prospects*. in *The Second International Oats Conference*. 1985. Aberystwyth, Wales: Martinus Nijhoff Publishers.

38. Robins, G., Pomeranz, Y., and Briggle, L., *Amino acid composition in oat groats* Journal of Agriculture and Food Chemistry, 1971. **19**: p. 536-539.

39. Pomeranz, Y., Youngs, V., and Robbins, G., *Protein content and amino acid composition of oat species and tissues.* Cereal Chemistry, 1973. **50**: p. 702-707.

40. Petersen, D. and Brinegar, C., *Oat storage proteins*, in *Oats: Chemistry and Technology*, Webster, F.H., Editor. 1986, American Association of Cereal Chemists Inc.: Minnesota.

41. Shewry, P., *Cereal grain proteins*, in *Cereal Grain Quality*, Henry, R.J. and Kettlewell, P.S., Editors. 1996, Chapman & Hall: London.

42. Laurian, S., Nozzolillo, C., Cudjoe, A., and Altosaar, I., *Total solublisation of groat proteins in high protein oat (avena sativa l. cv. hinoat): evidence that glutelins are a minor component.* Can. Inst. Food Sci. Technol., 1983. **16**(3): p. 196-200.

43. Kasarda, D., *Glutenin structure in relation to wheat quality*, in *Wheat is Unique*, Pomeranz, Y., Editor. 1989, American Association of Cereal Chemists: St Paul, Minnisota. p. 277-302.

44. Peterson, D., *Protein concentration, concentration of protein fractions and amino acid balance in oats.* . Crop Science, 1976. **16**: p. pages 663-6.

45. Draper, S., *Amino acid profiles of chemical and anatomical fractions of oat grains.* Journal of Science of Food and Agriculture, 1973. **24**: p. 1241-1250.

46. Youngs, V., *Oat lipids and lipid related enzymes*, in *Oats: Chemistry and Technology*, Webster, F.H., Editor. 1986, American Association of Cereal Chemists Inc.: Minnesota.

47. Molteberg, E., Vogt, G., Nilsson, A., and Frolich, W., *Effects of storage and heat processing on the content and composition of free fatty acids.* Cereal Chemistry, 1995. **72**(1): p. 88-93.

48. Gooding, M. and Davies, W., *Ethanol, starch and gluten production*, in *wheat production and utilization systems, quality and the environment*. 1997, CAB International: Oxon, UK.

49. Welch, R., *Oat milling processing and storage*, in *The Oat Crop Production and Utilisation*, Welch, R.W., Editor. 1995, Chapman & Hall: London.

50. Macdonald, R., Kim, E., and Morison, K. *The structure of gluten enriched oat dough evaluated by confocal laser scanning microscopy (CLSM).* in *Xth International Gluten Workshop (XIGW)*. 2009. Cleremont-Ferrand, France.

51. Al-Hakkak, J. and Al-Hakkak, F., *New Non-destructive method using gluten to isolate starch from plant materials other than wheat.* die starke, 2007. **59**: p. 117-124.

52. Al-Hakkak, J., *US 7001469: An improved procedure for separating starch from comminutates of plant materials*, in *United States Patent Office*, Office, U.S.P., Editor. 2006, New Zealand Institute for Crop and Food Research Limited: New Zealand.

53. Macdonald, R., Kim, E., and Morison, K. *CLSM evaluation of the structure of protein enriched oat dough*. in *8th World Congress of Chemical Engineering (WCCE8)*. 2009. Montreal.

54. Macdonald, R., Morgenstern, M., and Morison, K. *The effect of varying resting time on the large deformation characteristics of gluten enriched, sheeted oat dough*. in *Xth International Gluten Workshop (XIGW)*. 2009. Cleremont-Ferrand, France.

55. Macdonald, R., Morgenstern, M., and Morison, K. *Investigation of the effect of processing conditions on the rheology of sheeted oat gluten dough*. in *Chemeca 2009*. 2009. Perth, Australia.

56. van der zalm, E., van der Goot, A., and Boom, R., *Influence of process conditions on the separation behaviour of starch–gluten systems* Journal of Food Engineering, 2009. **95**(4): p. 572-578.

57. Peighambardoust, S. and van der Goot, A., *Migration of gluten under shear flow: Influence of process parameters on separation behaviour.* Food Chemistry, 2010. **118**: p. 712-718.

58. Peighambardoust, S., Hamer, R., Boom, R., and van der Goot, A., *Migration of gluten under shear flow as a novel mechanism for separating wheat flour into gluten and starch.* Journal of Cereal Science, 2007: p. 1-12.

59. Roels, S., Sindic, M., Deroanne, C., and Delcour, J., *Protein composition and agglomeration tendency of gluten isolated from European wheats (Triticum aetivum L) in a batter system.* Journal of Agriculture and Food Chemistry, 1998. **46**: p. 1344-1349.

60. Knight, J. and Olson, R., *Wheat starch: production, modification and uses*, in *Starch: Chemistry and Technology Industrial Aspects*, Whistler, R. and Paschall, E., Editors. 1984, Academic Press: New York. p. 491-506.

61. Frederix, S., Van Hoeymissen, K., Courtin, C., and Delcour, J.A., *Water-extractable and water-unextractable arabinoxylans affect gluten agglomeration behavior during wheat flour gluten-starch separation.* Journal of Agriculture and Food Chemistry, 2004. **52**: p. 7950-7956.

62. Yondem-Makascioglu, F., Dik, T., and Kincal, N., *An energy analysis of the manufacture of wheat starch and related products.* Journal of Science of Food and Agriculture, 1980. **31**: p. 299-307.

63. Auger, F., Morel, M., Dewilde, M., and Redl, A., *Mixing history affects gluten protein recovery, purity, and glutenin re-assembly capacity from optimally developed flour-water batters.* Journal of Cereal Science, 2009. **49**: p. 405-412.

64. Frederix, S., Courtin, C., and Delcour, J.A., *Influence of process parameters on yield and composition of gluten fractions obtained in a laboratory scale dough batter procedure.* Journal of Cereal Science, 2004. **39**: p. 29-36.

65. Dik, T., Yondem-Makascioglu, F., Aytac, C., and Kincal, N., *Wet separation of wheat flours into starch and gluten fractions: the combined effects of water to flour ratio-dough maturation time and the effects of flour aging and ascorbic acid addition.* Journal of Science of Food and Agriculture, 2002. **82**: p. 405-413.

66. Burnette, D., Lanz, M., Sisson, P., Sutherland, S., and Weaver, S., *Marketing, processing and uses of oat for food*, in *oat science and technology*, H G Marshall, M.E.S., Editor. 1992, American Society of Agronomy Inc: Wisconsin, USA. p. Chapter 9, Pages 247 - 263.

67. van der zalm, E., Swahalha, A., van der Goot, A., and Boom, R. *The separation of wheat flour into starch and gluten - Mechanisms and differences between flour cultivars*. in *5th International Symposium on Food Rheology and Structure*. 2009.

68. Yondem-Makascioglu, F., Dik, T., and Kincal, N., *Separation of bread wheat flours ito starch and gluten fractions: effect of water temperature alone or in combination with the water to flour ratio.* Journal of Science of Food and Agriculture, 2002. **82**: p. 414-420.

69. Frederix, S., Courtin, C., and Delcour, J.A., *Impact of xylanases with different substrate selectivity on gluten-starch separation of wheat flour.* Journal of Agriculture and Food Chemistry, 2003. **51**: p. 7338-7345.

70. Scholfield, J., *Wheat proteins: structure and functionality in milling and breadmaking*, in *Wheat Production, Properties and Quality*, Bushuk, W. and Rasper, V.F., Editors. 1994, Chapman & Hall: London.

71. Meuser, F., *Wheat utilization for the production of starch, gluten and extruded products*, in *Wheat Production, Properties and Quality*, Bushuk, W. and Rasper, V.F., Editors. 1994, Chapman & Hall: London.

72. Peighambardoust, S., *Development of dough under shear flow*, in *Food and Bioprocess Engineering Group*. 2006, University of Wageningen: Wageningen.

73. Oszvald, M., Tomoskozi, S., Tamas, L., and F, B., *The role of rice and added wheat protein in the mixing properties of different rice flours.* Acta Alimentaria, 2008(2008).

74. Lagrain, B., Thewissen, B., Brijs, K., and Delcour, J., *Mechanism of gliadin–glutenin cross-linking during hydrothermal treatment.* Food Chemistry, 2008. **107**: p. 753-760.

75. Manu, B. and Prasada Rao, U., *Influence of size distribution of proteins, thiol and disulfide content in whole wheat flour on rheological and chapati texture of Indian wheat varieties.* Food Chemistry, 2008. **110**: p. 88-95.

76. Larraque, O., Sharp, P., and Bekes, F. *Molecular weight distribution of gluten proteins*. in *Wheat production in stressed environments*. 2007. Argentina: Springer.

77. Robertson, G. and Cao, T., *Substitution of concentrated ethanol for water in the laboratory washing fractionation of protein and starch from hydrated wheat flour.* Cereal Chemistry, 1998. **75**: p. 508-513.

78. Weegels, P., Hamer, R., and Schofield, J., *Critical Review: functional properties of wheat glutenin.* Journal of Cereal Science, 1996. **23**(1): p. 1-17.

79. Hamer, R. and van Vleit, T. *Understanding the structure and properties of gluten: an overview*. in *Wheat Gluten - Proceeding of the Seventh International Workshop, Gluten 2000*. 2000. Cambridge: Royal Society of Chemistry.

80. Van Vliet, T. and Hamer, R. *Fourteen years strain hardening as an indicator of bread-baking performance, questions still to be solved*. in *9th International Gluten Workshop 2006*. 2006. San Francisco.

81. Don, C., Lichtendonk, W., Plijter, J., van Vliet, T., and Hamer, R., *The effects of mixing on glutenin particle properties: aggregation factors that effect gluten function in dough.* Journal of Cereal Science, 2005. **41**(2005): p. 69-83.

82. Don, C., Lichtendonk, W., Plijter, J., and Hamer, R., *The effects of dough mixing on the GMP re-aggregation and dough elasticity during dough rest*, in *The Gluten Proteins*, Lafiandra, D., Masci, S., and D'Ovidio, R., Editors. 2004, The Royal Society of Chemistry: Cambridge.

83. Belton, P., *Mini review on the elasticity of wheat gluten.* Journal of Cereal Science, 1999. **29**: p. 103-107.

84. Slotter, R. and Langford, C., *Wheat starch manufacture.* Industrial & Engineering Chemistry, 1944. **36**(5): p. 404-408.

85. Veal, E., *The effect of mixing at selected speeds and temperatures on the physical properties and baking behaviour of doughs made from a northwest wheat flour.*, in *Oregon State University*. 1954, Oregon State University.

86. Anderson, R., Pfeifer, V., and Lancaster, E., *Continuous batter process for separating gluten from wheat flour.* Cereal Chemistry, 1958. **35**: p. 449-457.

87. Burrington, K. *Adjusting Dough Rheology*. Food Product Design 2007 [cited 2003]; Available from: http://www.vpico.com/articlemanager/printerfriendly.aspx?article=77137.

88. GEA, M.S.D., *Starch from wheat*, in *Westfalia Separator Industry*. 2006, GEA Westfalia Separator: Germany.

89. Fellers, D. *Fractionation of wheat into major components*. in *Symposium Proceedings*. 1973. Missouri: AACC.

90. Conrad, E., *US 4282319: Process for the preparation of a hydrolysed product from whole grain*, in *United States Patent Office*, Office, U.S.P., Editor. 1981, Kockums Construction AB: USA.

91. Conrad, E., *US 4377602: Process for the preparation of a hydrolysed product from whole grain and such a product*, in *United States Patent Office*, Office, U.S.P., Editor. 1983, Nordstjernan AB: USA.

92. Nkonge, C. and Ballance, C., *Enzymatic solubilization of cereal proteins by commercial proteases.* Cereal Chemistry, 1984. **61**(4): p. 316-320.

93. Hohner, G. and Hyldon, R., *US 4028468: Oat groat fractionation process* in *United States Patent Office*, Office, U.S.P., Editor. 1977, The Quaker Oats Company: USA.

94. Davin, A., Godon, B., Nicolas, M., and Popineau, Y., *Incidneces varietals sur le rendement d'extraction et les carateristiques technologiques do gluten de ble.* Industries des cereales, 1984. **29**: p. 5-13.

95. Hamer, R., Weegels, P., Marseille, J., and Kelfkens, M., *A study of the factors effecting the separation of wheat flour into starch and gluten*, in *Wheat is Unique: Structure, Composition, Processing, Enduse Properties and Products*, Pomeranz, Y., Editor. 1989, AACC: Minnesota.

96. Kelfkens, M. and Hamer, R., *Agronomic factrors realted to the quality of wheat for the starch industry. Part 1: sprout damage.* die starke, 1991. **43**: p. 340-343.

97. Roels, S., *Factors governing wheat and wheat gluten functionality in breadmaking and gluten /starch separation*, in *Laboraotry of Food Chemistry*. 1997, Katholieke Universitheit Leuven: Leuven.

98. Saulnier, L., Andersson, R., and Aman, P., *A study of the polysaccharide components in gluten.* Journal of Cereal Science, 1997. **25**: p. 121-127.

99. Seguchi, M., Hayashi, M., Keanenaga, K., Ishihara, C., and Noguchi, S., *Springiness of pancake and its relation to the binding of prime starch to tailings in stored wheat flour.* Cereal Chemistry, 1998. **75**(1): p. 37-42.

100. Hoseney, R., *Principles of Cereal Science and Technology*. 2 ed. 1994, Minnesota: AACC.

101. Georgopoulos, T., Larsson, H., and Eliasson, A., *Influence of native lipids on the rheological properties of wheat flour dough and gluten.* Journal of texture studies, 2003. **37**: p. 46-62.

102. Guan, L., Seib, P., Graybosche, R., Bean, S., and Shi, Y., *Dough rheology and wet milling of hard waxy wheat flours.* Journal of Agriculture and Food Chemistry, 2009. **57**: p. 7030-7038.

103. Weegels, P., Marseille, J., and Hamer, R., *Enzymes as a processing aid in the separation of wheat flour into starch and gluten.* die starke, 1992. **44**: p. 44-48.

104. Wang, M., Oudgenoeg, G., van Vleit, T., and Hamer, R., *Interaction of water unextractable solids with gluten protein: effect on dough properties and gluten quality.* Journal of Cereal Science, 2003. **38**(2003): p. 95-104.

105. Angioloni, A. and Rosa, M., *Effects of cysteine and mixing conditions on white/whole dough rheological properties.* Journal of Food Engineering, 2007. **80**(1): p. 18-23.

106. Schurer, F., Kieffer, R., Wieser, H., and Koehler, P., *Effect of hydrostatic pressure and temperature on the chemical and functional properties of wheat gluten II. Studies on the influence of additives.* Journal of Cereal Science, 2006. **46**: p. 39-48.

107. Salvador, A., Sanz, T., and Fiszman, S., *Dynamic rheological characteristics of wheat flour–water doughs. Effect of adding NaCl, sucrose and yeast.* Food Hydrocolliods, 2006. **20**: p. 780-786.

108. Ranhotra, G. and Gelroth, J., *Food uses of oats*, in *The Oat Crop Production and Utilisation*, Welch, R.W., Editor. 1995, Chapman & Hall: London.

109. Paton, D., Bresciani, S., Han, N.F., and Hart, J., *Oats: chemistry, technology and potential uses in the cosmetic industry.* Cosmetics & Toiletries, 1995. **110**: p. 63-70.

110. Collins, F. and Paton, D., *US 5169660: Method of producing stable bran and flour products from cereal grains*, in *United States Patent Office*, Office, U.S.P., Editor. 1991, Exavena Oy: USA.

111. Cioca, G., Anderson, J., Cohen, I., Tadlock, C., and Bevacqua, A., *US 5552135: Sunscreens containing plant extracts*, in *United States Patent Office*, Office, U.S.P., Editor. 1996, The Quaker Oats Company: USA.

112. Evans, R., Jee, M., Sander, N., Smith, I., and Gibson, R., *US 5026548: Surfactant*, in *United States Patent Office*, Office, U.S.P., Editor. 1991, Cadbury Schweppes Plc: USA.

113. Redmond, M. and Fielder, D., *US 6818232: Oat extracts: refining, compositions and methods of use*, in *United States Patent Office*, Office, U.S.P., Editor. 2004, Ceapro, Inc: USA.

114. Oughton, R., *US 4211801: Process for the treatment of comminuted oats*, in *United States Patent Office*, Office, U.S.P., Editor. 1980, Du Pont of Canada Limited: USA.

115. Inglett, G., *US 4996063: Method for making a soluble dietary fibre composition from oats*, in *United States Patent Office*, Office, U.S.P., Editor. 1991, USA, Secretary of Agriculture: USA.

116. Inglett, G., *US 5082673: Method of making soluble dietary fibre compositions from cereals*, in *United States Patent Office*, Office, U.S.P., Editor. 1992, USA, Secretary of Agriculture: USA.

117. Targan, R., *US 5468491: Method for producing oat extract*, in *United States Patent Office*, Office, U.S.P., Editor. 1995: USA.

118. Barrows, V., Fulcher, R., and Paton, D., *US 4435429: Processing aqueous treated cereals*, in *United States Patent Office*, Office, U.S.P., Editor. 1984, Canadian patents and development limited: USA.

119. Ma, C. and Wood, D., *Functional properties of oat proteins modified by acylation, trypsin hydrolysis or linoleate treatment.* JAOCS, 1987. **64**(12): p. 1726-1731.

120. Ma, C., *Functional Properties of Oat Concentrate Treated With Linoleate or Trypsin.* Journal of the Institute of Canadian Science Technology, 1985. **18**(1): p. 79-84.

121. Lim, W., Liang, Y., Seib, P., and Rao, C., *Isolation of oat starch from oat flour.* Cereal Chemistry, 1992. **69**(3): p. 233-236.

122. Lenz, M. and Paisley, S., *US 5525151: Process for preparing a low fat, transparent, stable oat protein containing aqueous solution and product thereof*, in *United States Patent Office*, Office, U.S.P., Editor. 1996, The Quaker Oats Company: USA.

123. Malkki, Y., Myllymaki, O., and Tuomela, R., *US 5929231: Method for preparing fine-granuled and modified starches*, in *United States Patent Office*, Office, U.S.P., Editor. 1999, Exavena Oy: USA.

124. Sarkki, M., Soderstrom, M., Maunula, H., and Korhonen, A., *US 4448790: Process for fractionating grain flour into components of food quality*, in *United States Patent Office*, Office, U.S.P., Editor. 1984, Oy Vehna AB: USA.

125. Wu, Y., Sexton, K., Cluskey, J., and Inglett, G., *ProteiniIsolate from high protein oats:preparation, composition and properties.* Journal of Food Science, 1977. **42**(5): p. 1383-1386.

126. Wu, Y., Cluskey, J., Wall, J., and Inglett, G., *Oat protein concentrates from wet milling process : composition and properties.* Cereal Chemistry, 1973. **50**(4): p. 481-488.

127. Cluskey, J., Wu, Y., Wall, J., and Inglett, G., *Oat protein concentrates from wet milling process: preparation.* Cereal Chemistry, 1973. **50**(4): p. 481-488.

128. Bell, A., Boocock, J., and Oughton, R., *US 4089848: Extraction of protein food values from oats* in *United States Patent Office*, Office, U.S.P., Editor. 1978, Du Pont of Canada Limited: USA.

129. Buchi. *Buchi Mini Spray Drier B-290*. http://www.buchi.com/Mini-Spray-Dryer-B-290.179.0.html 2007 [cited 2008.

130. Macdonald, R., MacManus, R., and Morison, K. *SEM evaluation of the structure of dried oat starch granules*. in *Chemeca 2009*. 2009. Perth, Australia.

131. Macdonald, R., MacManus, R., and Morison, K. *SEM evaluation of the structure of dried oat starch granules*. in *8th World Congress of Chemical Engineering (WCCE8)*. 2009. Montreal.

132. Cardinali, M. and Lam, T. *New advances in starch-based particle technologies for aesthetic modification*. in *PCIA*. 2003. Manilla: national Starch Personal Care.

133. Dijkink, B., Sperenza, L., Paltsidis, D., and Vereijken, J., *Air dispersion of starch-protein mixtures: a predictive tool for air classisification performance.* Powder Technology, 2006. **172**: p. 113-119.

134. Vasanthan, T. and Temelli, F., *Grain fractionation technologies for cereal beta-glucan concentration.* Food Research International, 2008. **2008**(41): p. 876-881.

135. Lindeboom, L., Chang, P., and Tyler, R., *Analytical, biochemical and physicochemical aspects of starch granule size, with emphasis on small granule starches: a review.* die starke, 2004. **56**: p. 89-99.

136. Jane, J., Kasemsuwan, T., Leas, S., Zobel, H., and Robyt, J., *Anthology of starch granule morphology by scanning electron microscopy.* die starke, 1994. **46**(4): p. 121.129.

137. Chewangkul, L., Naivikul, O., Garnjanagoonchorn, W., Oates, C., Siroth, K., and Suwonsichon, T., *Microstructural changes in instant noodles during*

production via triple staining and confocal scanning microscopy and scanning electron microscopy. Jounral of Kasetsart (National Science), 2001. **35**: p. 311 - 317.

138. Durrenberger, M., Handschin, S., Conde-petit, B., and Escher, F., *Visualization of food structure by confocal scanning laser microscopy (cflm).* Lebensm.-Wiss. U. Technology, 2001. **PhD**(34): p. 11-17.

139. Lindeboom, L., *Studies on the characterization, biosynthesis and isolation of starch and protein from quinoa (Chenopodium Quinoa Wild),* in *Department of Applied Microbiology and Food Science*, Institute, I.S., Editor. 2005, University of Saskatchewan: Saskatoon.

140. Emami, S., *Hydrocyclone fractionation of chickpea flour and measurement of physical and functional properties of flour and starch and protein fractions*, in *Department of Agricultural and Bioresource Engineering*. 2007, University of Saskatchewan: Saskatoom.

141. Chmelik, *Comparison of size characterization of barley starch granules determined by electron and optical microscopy, low angle laser light scattering and gravitational fieldflow fractionation.* Journal Inst. Brewing, 2001. **107**: p. 11-17.

142. Raeker, M., Gaines, S., Finney, P., and Donelson, T., *Granule size distribution and chemical composition of starches from 12 soft wheat cultivars.* Cereal Chemistry, 1998. **75**: p. 721-728.

143. Sajeev, M. and Kailappan, R., *Studies on settling of cassava starch - Part I: Gravity settling.* Journal of Root Crops, Indian Society for Root Crops, 2005.

144. Engineering, D.o.M. *JEOL JSM 7000F Field Emission Gun Scanning Electron Microscope*. http://www.mech.canterbury.ac.nz/labs/materials.shtml 2007 [cited 2007.

145. Walton, D. and Mumford, C., *Spray dried products - characterisation of particle morphology.* Trans IChemE, 1999. **77**(A): p. 21-38.

146. Wilhelm, E., Aberle, T., Burchard, W., and Landers, R., *Peculiarities of aqueous amaranth starch suspensions.* Biomacromolecules, 2002. **2002**(3): p. 17-26.

147. Bergthaller, W., Kersting, H., Varavinit, S., and Wongsagonsup, R. (2008) *Spray-dried rice starch - parameters of agglomerate formation and potential utilisation in tableting*. Association of Cereal Research.

148. Atichokudomchai, A., Shobsngob, S., and Varaninit, S., *Morphological Properties of Acid-modificed Tapioca Starch.* die starke, 2000. **52**: p. 283-289.

149. Puchongkavarin, H., Bergthaller, W., Shobsngob, S., and Varaninit, S., *Characterisation of utilization of acid-modified rice starches for use in pharmaceutical table compression.* die starke, 2003. **55**: p. 464-475.

150. Zhao, J. and Whistler, R., *Isolation and characterisation of starch from amaranth flour.* Cereal Chemistry, 1994. **71**(4): p. 392-393.

151. Varaninit, S. and Mitrevej, A., *Production of spray dried rice starch and its utilization in the pharmaceutical industry.* Microbial Utilization of Renewable Resources, 1989. **6**: p. 158-162.

152. Grace, M., *Cassava Processing*. FAO Plant production and protection series. Vol. 3. 1977, Rome: Food and Agriculture Organisation of the United Nations.

153. Sajeev, M., Moorthy, S., Kailappan, R., and Rani, V., *Gelatinisation characteristics of cassava starch settled in the presence of different chemicals.* die starke, 2003. **55**: p. 213-221.

154. Tester, R., Karkalas, J., and Qi, X., *Starch—composition, fine structure and architecture.* Journal of Cereal Science, 2003. **39**: p. 151.165.

155. Tanner, R., *Engineering Rheology*. 2 ed. Oxford Engineering Science Series, ed. Tanner, R. Vol. 52. 2000: Oxford University Press.

156. Dobraszczyk, B. and Morgenstern, M., *Rheology and the breadmaking process.* Journal of Cereal Science, 2003. **38**: p. 229-245.

157. Kokelaar, *Physics of Breadmaking*, in *Food and Bioprocess Engineering Group*. 1994, University of Wageningen: Wageningen.

158. Stojceska, V., Butler, F., Gallagher, E., and Keehan, D., *A comparison of the ability of several small and large deformation rheological measurements of wheat dough to predict baking behaviour.* Journal of Food Engineering, 2007. **83**(4): p. 475-482.

159. Smith, W., *Foundations of Materials Science and Engineering*, . Second ed. 1993, Singapore: McGraw-Hill Ltd.

160. Bloksma, A.H. and Bushuk, W., *Rheology and chemistry of dough*, in *Wheat: Chemistry and Technology*, Pomeranz, Y., Editor. 1988, American Association of Cereal Chemists: Minnesota.

161. Morgenstern, M., Newberry, M., and Holst, S., *Extensional properties of dough sheets.* Cereal Chemistry, 1996. **73**(4): p. 478-482.

162. Holland, F. and Bragg, R., *Fluid Flow for Chemical Engineers*. 2 ed. 1995, London: Edward Arnold.

163. Georgopoulos, T., Larsson, H., and Eliasson, A., *A comparison of the rheological properties of wheat flour dough and its gluten prepared by ultracentrifugation.* Food Hydrocolliods, 2003. **18**: p. 143-151.

164. Shuey, W.C., *Practical instruments for rheological measurements on wheat products.* Cereal Chemistry, 1975. **52**: p. 42-81.

165. Tronsmo, K., Magnus, E., Baardseth, P., Schofield, J., Aamodt, A., and Faergestad, E., *Comparison of small and large deformation rheological properties of wheat dough and gluten.* Cereal Chemistry, 2003. **80**(5): p. 578-595.

166. Muller, H., *Rheology and the conventional bread and biscuit making process.* Cereal Chemistry, 1975. **52**: p. 89-105.

167. Rozzi, L., *Rheological properties of bread flour at elevated temperatures*. 2007, Purdue University.

168. Angioloni, A., Balestra, F., Pinnavaia, G., and Rosa, M., *Small and large deformation tests for the evaluation of frozen dough viscoelastic behaviour.* Journal of Food Engineering, 2008. **87**: p. 527-531.

169. Kieffer, R., Wieser, H., Henderson, M.H., and Graveland, A., *Correlations of the breadmaking performance of wheat flour with rheological measurements on the micro-scale.* Journal of Cereal Science, 1998. **27**: p. 53-60.

170. Dobraszczyk, B. and Roberts, C.A., *Strain hardening and dough gas cell-wall failure in biaxial extension.* Journal of Cereal Science, 1994. **20**: p. 265-274.

171. Van Vliet, T., Janssen, A.M., Bloksma, A.H., and Walstra, P., *Strain hardening of dough as a requirement for gas retention.* Journal of Texture Studies, 1992. **23**(4): p. 439 - 460.

172. Van Vliet, T., *Strain Hardening as an indicator of bread-making performance: A review with discussion.* Journal of Cereal Science, 2008. **48**: p. 1-9.

173. Semiatin, S., ed. *ASM handbook. 14B. Metalworking: Sheet Forming*. Trans. AIME, ed. Committee, A.I.H. 2006, ASM International.

174. Kieffer, R., *Studies on the differing properties of gluten and dough on the formation of the gluten network,* in *The Gluten Proteins*, Lafiandra, D., Masci, S., and D'Ovidio, R., Editors. 2004, The Royal Society of Chemistry: Cambridge.

175. Sutton, K., Larsen, N., Morgenstern, M., Ross, M., Simmons, L., and Wilson, A., *Differing effects of mechanical dough development on sheeting development methods and aggregated glutenin proteins.* Cereal Chemistry, 2003. **80**(6): p. 707-158.

176. Perez, G., Ribotta, P., Steffolani, M., and Leon, A., *Effect of soybean protein on gluten depolymerisation during mixing and resting.* Journal of Food Science and Agriculture, 2008. **88**: p. 155*463.

177. Fustier, P., Castaigne, F., Turgeon, S.L., and Biliaderis, C.G., *Flour constituent interactions and their influence on dough rheology and quality of semi-sweet biscuits: A mixture design approach with reconstituted blends of gluten, water-solubles and starch fractions.* Journal of Cereal Science, 2007. **48**: p. 144-158.

178. Zheng, H., Morgenstern, M., Campanella, O., and Larsen, N., *Rheological properties of dough during mechanical dough development.* Journal of Cereal Science, 2000. **32**: p. 293-306.

179. Wilson, A., Morgenstern, M., and Kavale, S., *Mixing response of a variable speed 125g laboratory scale mechanical dough development mixer.* Journal of Cereal Science, 2001. **34**: p. 151-158.

180. Auger, F., Redl, J., Lefebvrre, J., and Morel, M. *On the mechanism of gluten network formation in flour-water batter dough*. in *The Gluten Proteins 2006*. 2006.

181. Every, D., Griffin, W., Simmins, L., and Sutton, K., *Ascorbate improver effects, dough mixing properties and bread quality interdependence on flour protein composition*, in *The Gluten Proteins*, Lafiandra, D., Masci, S., and D'Ovidio, R., Editors. 2004, The Royal Society of Chemistry: Cambridge. p. 341-344.

182. Larsson, H. and Hedlund, U., *On the swelling properties of gluten in the presence of salts and reduced pH*, in *The Gluten Proteins*, Lafiandra, D., Masci, S., and D'Ovidio, R., Editors. 2004, The Royal Society of Chemistry: Cambridge.

183. Ren, D., Walker, C.E., and Faubion, J.M., *Review - Correlating dough elastic recovery during sheeting with flour analysis and rheological properties.* Journal of Science of Food and Agriculture, 2008. **88**: p. 2581-2588.

184. Wang, M., van Vleit, T., and Hamer, R., *Effect of pentosans on gluten formation and properties*, in *The Gluten Proteins*, Lafiandra, D., Masci, S., and D'Ovidio, R., Editors. 2004, The Royal Society of Chemistry: Cambridge. p. 361-364.

185. Peighambardoust, S., van Brenk, S., van der Goot, A., Hamer, R., and Boom, R., *Dough processing in a couette-type device with varying eccentricity: effect on glutenin macro-polymer properties and dough micro-structure.* Journal of Cereal Science, 2007. **45**: p. 34-48.

186. Robertson, G., Gregorski, K., and Cao, T., *Changes in secondary protein structures during mixing development of high absorption (90%) flour and water mixtures.* Cereal Chemistry, 2006. **83**(2): p. 136-142.

187. van der Goot, A., Peighambardoust, S., Akkermans, C., and van Oosten-Manski, J., *Creating novel structures in food materials: the role of well defined shear flow*. Food Biophysics, 2008. **3**: p. 120 - 125.

188. Weegels, P., van de Pijpekamp, A., Graveland, A., Hamer, R., and Schofield, J., *Depolymerisation and re-polymerisation of wheat glutenin during dough processing. i. relationships between glutenin macropolymer content and quality parameters.* Journal of Cereal Science, 1996. **23**(2): p. 103-111.

189. Sutton, K., Simmons, L., Morgenstern, M., Chen, A., and Crocker, T. *Dough gas cell structure imaged by confocal microscopy*. in *RACI Cereal Chemistry Conference*. 2001.

190. Liu, G., Li, J., Shi, K., Wang, S., Chen, J., Liu, Y., and Huang, Q., *Composition, secondary structure, and self-assembly of oat protein isolate.* Journal of Agriculture and Food Chemistry, 2009. **57**: p. 4552-4558.

191. Sissons, M., Soh, H., and Turner, M., *Role of gluten and its components in influencing durum wheat dough properties and spaghetti cooking quality.* Journal of Food Science and Agriculture, 2008. **87**: p. 1874-1885.

192. Larroque, O., Gianibelli, M., Batey, I., and MacRitchie, F., *Electrophoretic characterisation of fractions collected from gluten protein extracts subjected to size-exclusion hgh-performacne liquid chromotography.* Electrophoresis, 1997. **18**: p. 1064-1067.

193. Malumba, P., Vanderghem, C., Deroanne, C., and Bera, F., *Influence of drying temperature on the solubility, the purity of isolates and the electrophoretic patterns of corn proteins.* Food Chemistry, 2008. **111**: p. 564-572.

194. Mohamed, A., Biresaw, G., Xu, J., Hojilla-Evangelista, M., and Rayas-Duarte, P., *Oats protein isolate: Thermal, rheological, surface and functional properties.* Food Research International, 2009. **42**: p. 107-114.

195. Donhowe, E. and Petersen, D., I*solation and characterization of oat aleurone and starchy endosperm protein bodies.* Plant Physiology, 1983. **71**: p. 519-523.

196. Wieser, H., *Chemistry of gluten proteins.* Food Microbiology, 2007. **24**: p. 115-119.

197. Visschers, R. and de Jonge, H., *Disulphide bond formation in food protein aggregation and gelation.* Biotechnolgy Advances, 2005. **23**: p. 75-80.

198. Maforimbo, E., G, S., Uthayakumanran, S., and Wrigley, C., *Incorporation of soy proteins into wheat-gluten matric during dough mixing.* Journal of Cereal Science, 2008. **47**(2008): p. 380-385.

199. Bulaj, G., *Formation of disulphide bonds in proteins and peptides.* Biotechnology Advances, 2005. **23**: p. 87-92.

200. Pareyt, B., Brijs, K., and Delcour, J.A., S*ugar-snap cookie dough setting: the impact of sucrose on gluten functionality.* Journal of Agriculture and Food Chemistry, 2009. **57**: p. 7814-7818.

201. Edwards, N., Mulvaney, S., Scanlon, M., and Dexter, J., R*ole of gluten and its components in determining durum semonlina dough viscoelastic properties.* Cereal Chemistry, 2003. **6**(80): p. 755-763.

202. Uthayakumanran, S., Stoddard, P., Gras, P., and Bekes, F., *Optimized methods for incorportating glutenin subunits into wheat dough for extension and baking studies.* Cereal Chemistry, 2000. **77**: p. 731-736.

203. Cubadda, R., Carcea, M., Marconi, E., and Trivisonno, M., *Influence of protein content on durum wheat gluten strength determined by the SDS sedimentation test and by other methods.* Cereal Foods World, 2007. **52**(5): p. 273-277.

204. Lorimer, N., Zabik, E., Harte, B., Stachiw, C., and AM, U., *Effect of navy bean protein flour and bean globulins on composition flour rheology, chemical bonding and microstructure.* Cereal Chemistry, 1991(68): p. 213-220.

205. Online, P. *SDS-PAGE* 2010 [cited; Available from: http://www.protocol-online.org/prot/Molecular_Biology/Protein/Protein_Electrophoresis/SDS-PAGE/index.html.

206. Foods, A.S., *Cereal flour prices*. 2010.

207. Earle, R. and Earle, M., *Mixing*, in *Unit Operation in Food Processing*, Earle, R., Editor. 1983, NZIFST (Inc): Wellington, NZ.

208. Perry, R. and Green, D., *Perry's Chemical Engineers Handbook - 8th Edition*. 8 ed, ed. Perry, R. and Green, D. 2008, New York: McGraw-Hill.

209. Miller, R. and Hoseney, R., *Role of salt in baking.* Cereal Foods World, 2008. **53**(1): p. 4-6.

210. van der zalm, E., van der Goot, A., and Boom, R., *The influence of sodium chloride on shear flow induced starch-gluten separation from Siossons wheat dough.* Journal of Food Engineering, 2010(99): p. 366-372.

211. Ukai, T., Matsumura, Y., and Urade, R., *Disaggregation and reaggregation of gluten proteins by sodium chloride.* Journal of Agriculture and Food Chemistry, 2008. **56**: p. 122-1130.

212. Larsson, H. and Eliasson, A., *Phase Separation of wheat flour dough studies by ultrcentrifugation and stress relaxation II. Influence of mixing time, ascorbic acid, and lipids.* Cereal Chemistry, 1996(73): p. 25-31.

12. Appendix A

Matrix Comparison of Tray Dried Starch Samples

Table A. Matrix Comparison of the Appearance of the Different Dried Samples at Low Magnification (X500).

Sample		**A**	**B**	**C**	**D**	**H**
	CODE	**W1-0 SD**	**W1-1 SD**	**W2-0 SD**	**W1-0A SD**	**W1-1 TD_M**
A	W1-0 SD	A has many starch granule agglomerates are visible as roughly spherical composite particles, ranging in diameter from 15 µm to over 30µm. A has few individual starch granules visible. Some protein agglomerates visible in A as irregularly shaped particles.	A has more defined roughly spherical agglomerates of starch granules, compared to B.	A is similar in appearance to C	D has more defined roughly spherical agglomerates of starch granules, compared to A.	H has a very different appearance to A, with obviously smaller particles present and no spherical agglomerates.
B	W1-1 SD		B has predominantly individual starch granules are visible. The few starch granules aggregates that are present in B are irregularly shaped and typically less than 15	C has more defined roughly spherical agglomerates of starch granules, compared to B.	D has more defined roughly spherical agglomerates of starch granules, compared to A.	H has a finer appearance to B, with obviously smaller particles present.

Sample		**A**	**B**	**C**	**D**	**H**
	CODE	**W1-0 SD**	**W1-1 SD**	**W2-0 SD**	**W1-0A SD**	**W1-1 TD_M**
			µm. Some protein agglomerates visible as irregularly shaped particles in B.			
C	W2-0 SD			C has some starch granule aggregates are visible. Many are roughly spherical in shape, ranging in diameter from 15 µm to over 30 µm. C has some individual starch granules visible. Some protein agglomerates visible as irregularly shaped particles in C.	D has more defined roughly spherical agglomerates of starch granules, compared to A.	H has a very different appearance to C, with obviously smaller particles present and no spherical agglomerates.
D	W1-0A SD				D has predominantly starch granule aggregates are visible as roughly spherical composite particles, ranging in diameter from 15 µm to over 30 µm. D has virtually no individual starch	H has a very different appearance to D, with obviously smaller particles present and no spherical agglomerates. There agglomerates present which are very irregular in size and shape.

Sample		A	B	C	D	H
	CODE	**W1-0 SD**	**W1-1 SD**	**W2-0 SD**	**W1-0A SD**	**W1-1 TD_M**
					granules visible. Some protein agglomerates visible as irregularly shaped particles in D.	
H	W1-1 TD_M	-	-	-	-	No starch granule aggregate are present in H. Many small irregularly shaped particles are present in H, which are adhered to the surface of larger starch granules. Overall H has a finer appearance

Table B. Matrix Comparison of the Appearance of the Different Dried Samples at High Magnification (X2500 and X5000).

Sample		**A**	**B**	**C**	**D**	**H**
	CODE	**W1-0 SD**	**W1-1 SD**	**W2-0 SD**	**W1-0A SD**	**W1-1 TD**
A	W1-0 SD	A has roughly spherical, well formed, tightly packed, starch granule agglomerates, ranging in diameter from 15 µm to over 30µm. A few individual starch granules and smaller and more loosely packed are visible. Some small particles <1µm diameter are stuck to the surface of larger granules. Some bridging between starch granules in agglomerates is visible	B has fewer and smaller starch granule agglomerates compared to A	C is similar in appearance to A.	Starch granule aggregates in D are similar in size and shape to A, but are more tightly formed and better defined. There are fewer individual starch granules compared to A.	Compared to A, H has considerably more damage to the starch granules (fractures and gouges). H has considerably more small particles (fractured starch granules). H does not have roughly spherical agglomerates, like A.
B	W1-1 SD		Only small starch granule agglomerates are visible, comprising two or three individual granules. Many individual starch	C has more and larger starch granule agglomerates compared to B.	D has more and larger starch granule agglomerates compared to B.	Compared to B, H has considerably more damage to the starch granules (fractures and gouges). H has considerably

Sample		A	B	C	D	H
	CODE	**W1-0 SD**	**W1-1 SD**	**W2-0 SD**	**W1-0A SD**	**W1-1 TD**
			granules are visible. Some bridging between starch granules in agglomerates is visible			more small particles (fractured starch granules).
C	W2-0 SD			C has roughly spherical, well formed, tightly packed, starch granule agglomerates, ranging in diameter from 15 µm to over 30µm. A few individual starch granules and smaller and more loosely packed are visible. Some small particles <1µm diameter are stuck to the surface of larger granules. Some bridging between starch granules in agglomerates is visible.	Starch granule aggregates in D are similar in size and shape to C, but are more tightly formed and better defined. There are fewer individual starch granules compared to C.	Compared to C, H has considerably more damage to the starch granules (fractures and gouges). H has considerably more small particles (fractured starch granules). H does not have roughly spherical agglomerates, like D.
D	**W1-1 TD**				D has roughly spherical, well formed, tightly packed, starch	Compared to D, H has considerably more damage to the starch

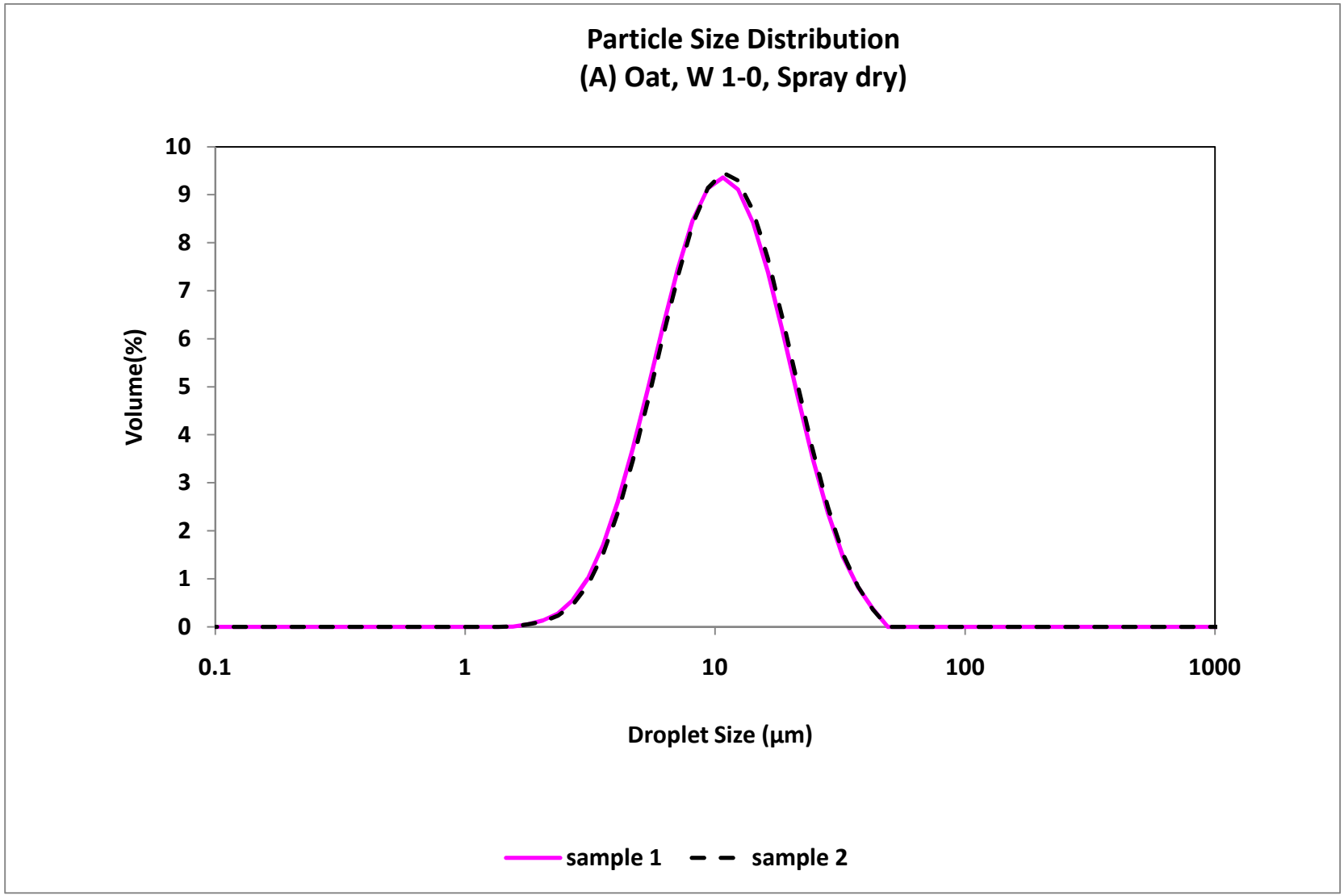
Sample		A	B	C	D	H
	CODE	**W1-0 SD**	**W1-1 SD**	**W2-0 SD**	**W1-0A SD**	**W1-1 TD**
					granule agglomerates, ranging in diameter from 15 µm to over 30µm. D has virtually no individual starch granules visible. Some of the larger individual granules >7µm appear mis-shapen as if softened and deformed. Small particles <1µm diameter are stuck to the surface of larger granules. Bridging between starch granules in agglomerates is visible.	granules (fractures and gouges). H has considerably more small particles (fractured starch granules). H does not have roughly spherical agglomerates, like D.
H	**W2-0 SD**					H has some irregularly shaped starch granule agglomerates. H has some individual starch granules visible. Many of the larger

Sample		**A**	**B**	**C**	**D**	**H**
	CODE	**W1-0 SD**	**W1-1 SD**	**W2-0 SD**	**W1-0A SD**	**W1-1 TD**
						individual starch granules >7µm have fracture lines and gouges. H has a large number of small, <1µm diameter, irregularly shaped particles (fractured starch granules) which are stuck to the surface of larger starch granules.

Matersizer Results

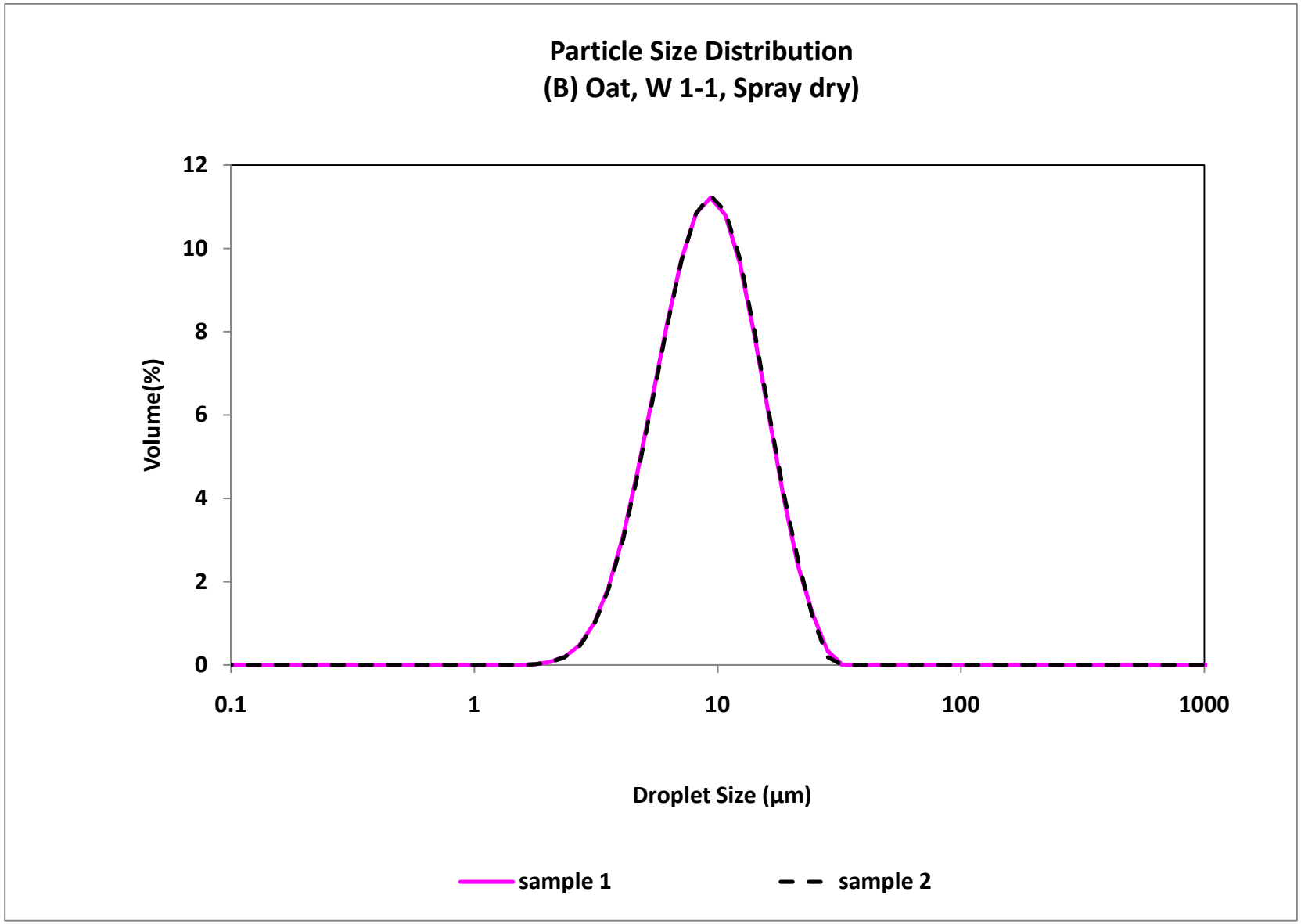
Mastersizer Result Sample A

Sample Name	Sample 1	Sample 2
Sample Name	A-Oat starch, W 1-0, spray dry	A-Oat starch, W 1-0, spray dry
Measurement date and time	Wednesday, 22 October 2008 3:42:59 p.m.	Wednesday, 22 October 2008 3:58:27 p.m.
Analysis date and time	Wednesday, 22 October 2008 3:43:01 p.m.	Wednesday, 22 October 2008 3:58:28 p.m.
Particle name	starch	starch
Particle refractive index	1.5	1.5
Particle absorption index	0	0
Dispersant name	Water	Water
Dispersant refractive index	1.33	1.33
Accessory name	Hydro 2000S (A)	Hydro 2000S (A)
Analysis model	General purpose	General purpose
Start result channel size	0.02	0.02
Last result channel size	2000	2000
Result emulation	Off	Off
Obscuration	11.71	12.61
Residual	0.688	0.497
Concentration	0.0144	0.016
Result transform type	Volume	Volume
Uniformity	0.496	0.486
Specific surface area	0.671	0.654
d (0.1)	5.004	5.164
d (0.5)	10.561	10.816
d (0.9)	21.854	22.136
D [3, 2] - Surface weighted mean	8.938	9.172
D [4, 3] - Volume weighted mean	12.253	12.482


Particle Size Distribution
(A) Oat, W 1-0, Spray dry)
Volume(%)
10
9
8
7
6
5
4
3
2
1
0
0.1
1
10
100
1000
Droplet Size (µm)
sample 1
sample 2

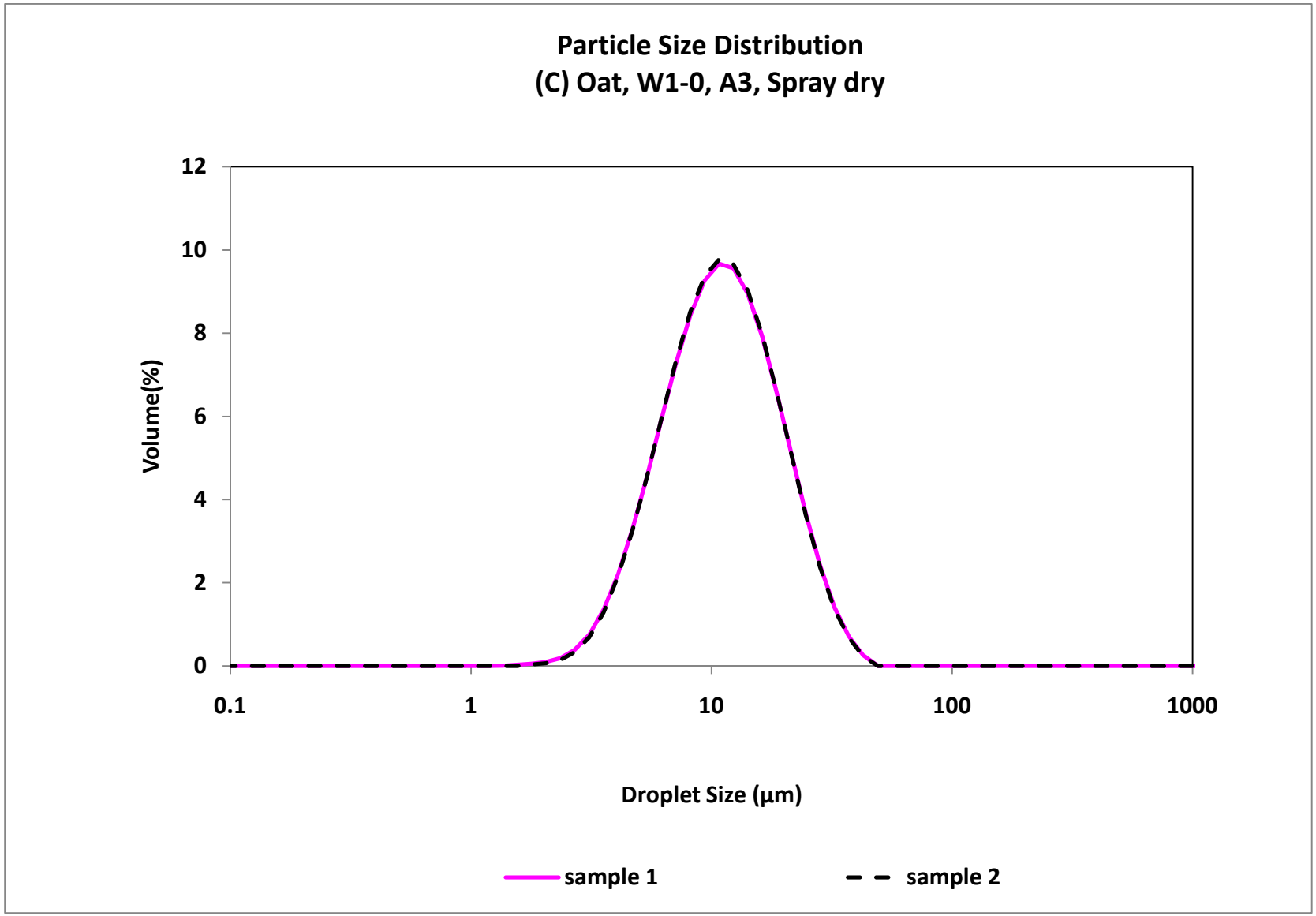
Mastersizer Result Sample B

Sample Name	sample 1	sample 2
Sample Name	B-Oat starch, W 1-1, spray dry	B-Oat starch, W 1-1, spray dry
Measurement date and time	Wednesday, 22 October 2008 4:08:04 p.m.	Wednesday, 22 October 2008 4:12:13 p.m.
Analysis date and time	Wednesday, 22 October 2008 4:08:05 p.m.	Wednesday, 22 October 2008 4:12:14 p.m.
Particle name	starch	starch
Particle refractive index	1.5	1.5
Particle absorption index	0	0
Dispersant name	Water	Water
Dispersant refractive index	1.33	1.33
Accessory name	Hydro 2000S (A)	Hydro 2000S (A)
Analysis model	General purpose	General purpose
Start result channel size	0.02	0.02
Last result channel size	2000	2000
Result emulation	Off	Off
Obscuration	11.7	13.75
Residual	0.714	0.721
Concentration	0.013	0.0155
Result transform type	Volume	Volume
Uniformity	0.397	0.392
Specific surface area	0.746	0.742
d (0.1)	4.837	4.872
d (0.5)	9.095	9.145
d (0.9)	16.486	16.504
D [3, 2] - Surface weighted mean	8.038	8.082
D [4, 3] - Volume weighted mean	10.008	10.031


Particle Size Distribution
(B) Oat, W 1-1, Spray dry)
12
10
8
6
4
2
0
Volume(%)
0.1
1
10
100
1000
Droplet Size (µm)
sample 1
sample 2

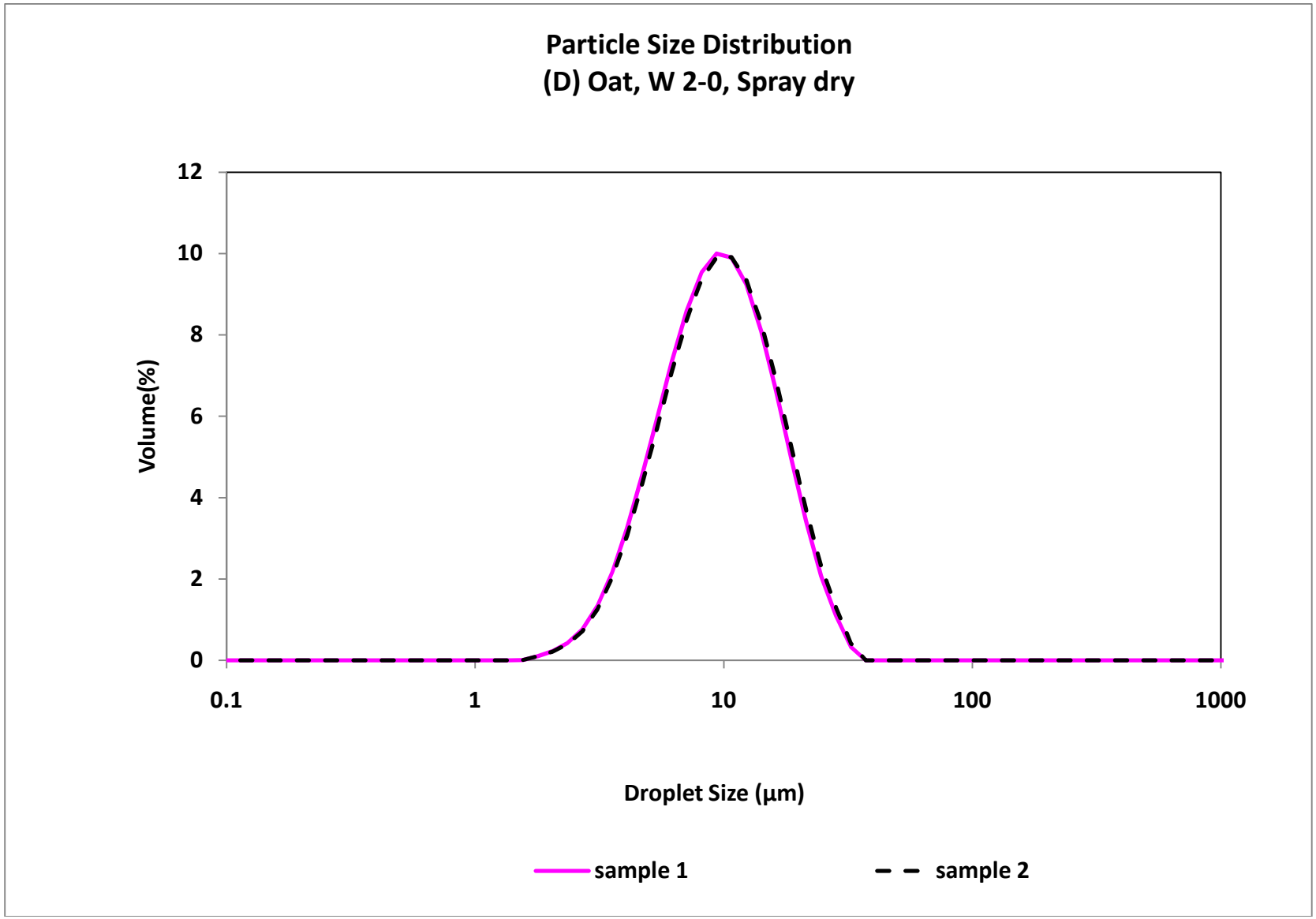
Mastersizer Result Sample C

Sample Name	sample 1	sample 2
Sample Name	C-Oat starch, W 1-0, A 3, spray dry	C - Oat starch, W 1-0, A3 spray dry
Measurement date and time	Wednesday, 22 October 2008 4:42:51 p.m.	Wednesday, 22 October 2008 4:47:59 p.m.
Analysis date and time	Wednesday, 22 October 2008 4:42:52 p.m.	Wednesday, 22 October 2008 4:48:00 p.m.
Particle name	starch	starch
Particle refractive index	1.5	1.5
Particle absorption index	0	0
Dispersant name	Water	Water
Dispersant refractive index	1.33	1.33
Accessory name	Hydro 2000S (A)	Hydro 2000S (A)
Analysis model	General purpose	General purpose
Start result channel size	0.02	0.02
Last result channel size	2000	2000
Result emulation	Off	Off
Obscuration	19.08	10.88
Residual	0.476	0.503
Concentration	0.0256	0.0141
Result transform type	Volume	Volume
Uniformity	0.471	0.466
Specific surface area	0.645	0.64
d (0.1)	5.291	5.357
d (0.5)	10.933	10.944
d (0.9)	21.875	21.764
D [3, 2] - Surface weighted mean	9.3	9.38
D [4, 3] - Volume weighted mean	12.492	12.485


Particle Size Distribution
(C) Oat, W1-0, A3, Spray dry
12
10
8
6
4
2
0
Volume(%)
0.1
1
10
100
1000
Droplet Size (µm)
sample 1
sample 2

Mastersizer Result Sample D

Sample Name	sample 1	sample 2
Sample Name	D - Oat starch, W 2-0, spray dry	D - Oat starch, W 2-0, spray dry
Measurement date and time	Wednesday, 22 October 2008 4:53:32 p.m.	Wednesday, 22 October 2008 4:56:56 p.m.
Analysis date and time	Wednesday, 22 October 2008 4:53:33 p.m.	Wednesday, 22 October 2008 4:56:57 p.m.
Particle name	starch	starch
Particle refractive index	1.5	1.5
Particle absorption index	0	0
Dispersant name	Water	Water
Dispersant refractive index	1.33	1.33
Accessory name	Hydro 2000S (A)	Hydro 2000S (A)
Analysis model	General purpose	General purpose
Start result channel size	0.02	0.02
Last result channel size	2000	2000
Result emulation	Off	Off
Obscuration	15.04	14.95
Residual	0.561	0.56
Concentration	0.0168	0.017
Result transform type	Volume	Volume
Uniformity	0.447	0.45
Specific surface area	0.745	0.73
d (0.1)	4.625	4.711
d (0.5)	9.436	9.654
d (0.9)	18.237	18.711
D [3, 2] - Surface weighted mean	8.052	8.217
D [4, 3] - Volume weighted mean	10.592	10.841


Particle Size Distribution
(D) Oat, W 2-0, Spray dry
Volume(%)
12
10
8
6
4
2
0
0.1
1
10
100
1000
Droplet Size (µm)
sample 1
sample 2

13. Appendix B

Supporting Rheological Data

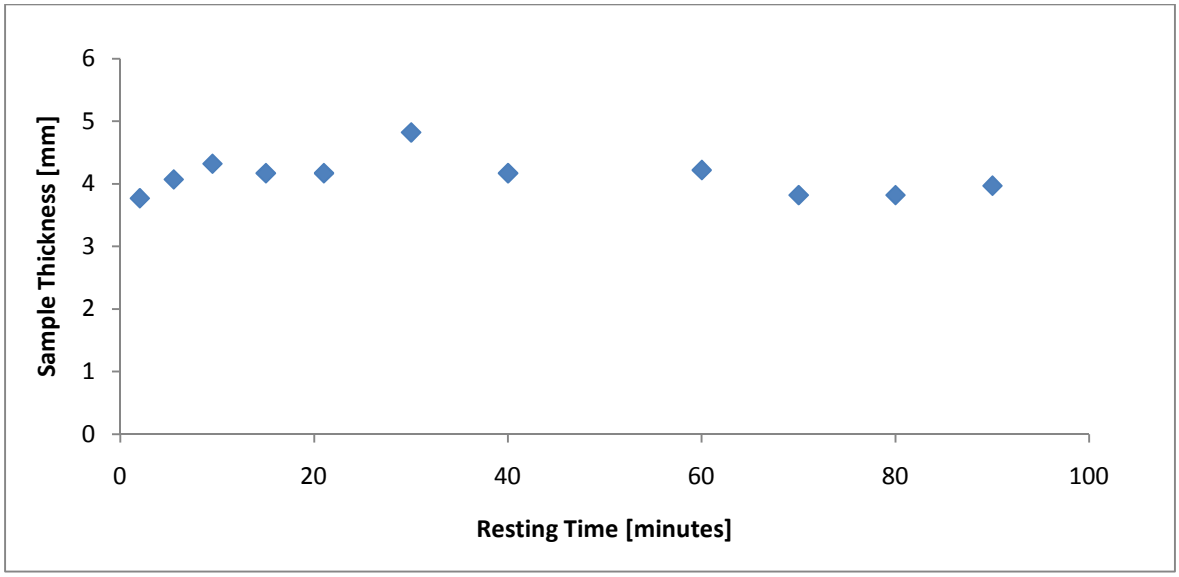


Figure B.1. Sample thickness for samples processed using standard conditions with different resting times

Table B.1. Strain Hardening and Apparent Modulus of Elasticity at *e*=1 - Resting Time

Resting time (minutes)	Strength index (*K*)	Strain hardening index (*n*)	Apparent modulus of elasticity (E_a)
0	12.2	1.55	15.5
2	16.1	1.60	19.8
5.5	15.2	1.60	18.2
9.5	14.2	1.60	17.2
15	14.7	1.64	18.3
21	13.8	1.56	16.7
30	12.0	1.62	15.3
40	12.6	1.60	16.6
60	12.9	1.56	16.3
70	13.1	1.57	16.8
80	12.8	1.53	15.7
90	12.3	1.53	14.7

Table B.2. Processing Parameters at Sample Failure for Various Resting Times

Resting time (minutes)	**Stress (σ)**	**Strain (e)**	**Strain rate ($\dot{e}$)**	**Apparent modulus of elasticity (E_a)**
0	20.0	1.29	0.021	15.5
2	22.4	1.13	0.028	19.8
5.5	21.7	1.19	0.023	18.2
9.5	21.4	1.24	0.022	17.2
15	22.8	1.24	0.022	18.3
21	20.6	1.24	0.022	16.6
30	19.7	1.29	0.024	15.3
40	22.2	1.34	0.023	16.6
60	21.2	1.30	0.021	16.3
70	22.4	1.33	0.023	16.8
80	19.9	1.26	0.022	15.7
90	18.2	1.23	0.022	14.7

14. Appendix C

Confocal Scanning Laser Microscope Images

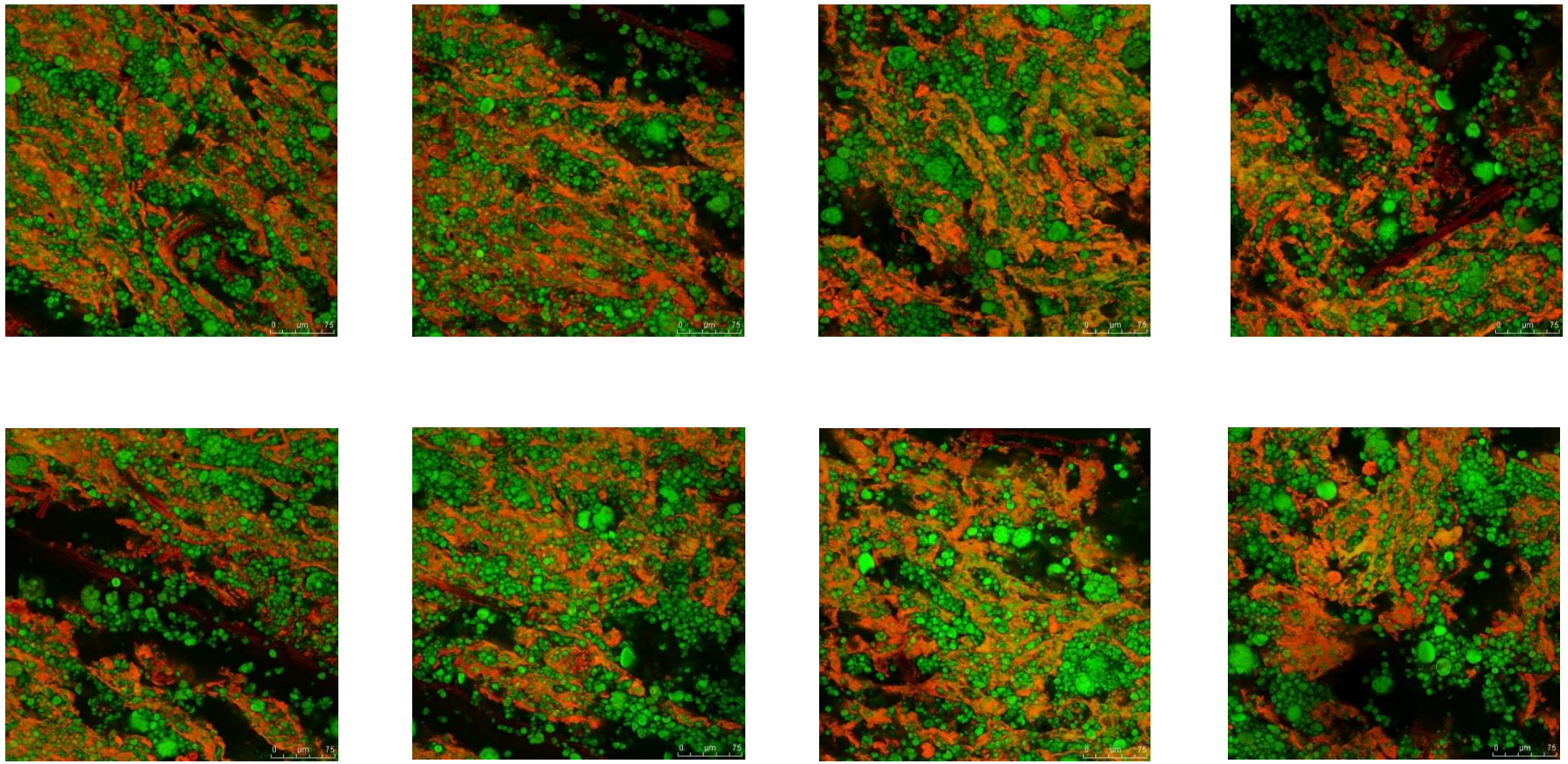


Figure C.1. Sample 14 Confocal Scanning Laser Microscope Images

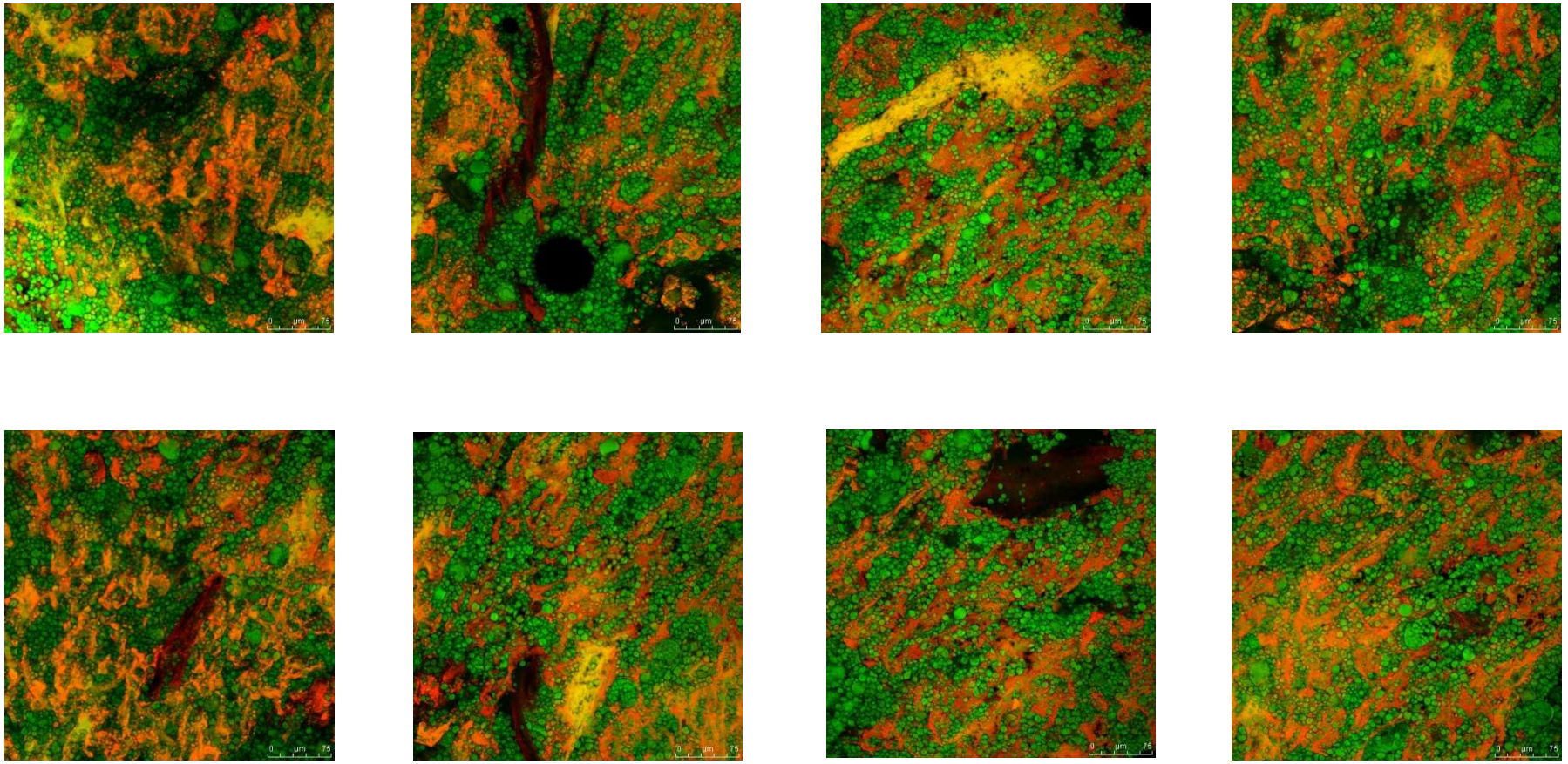


Figure C.2. Sample 15 Confocal Scanning Laser Microscope Images

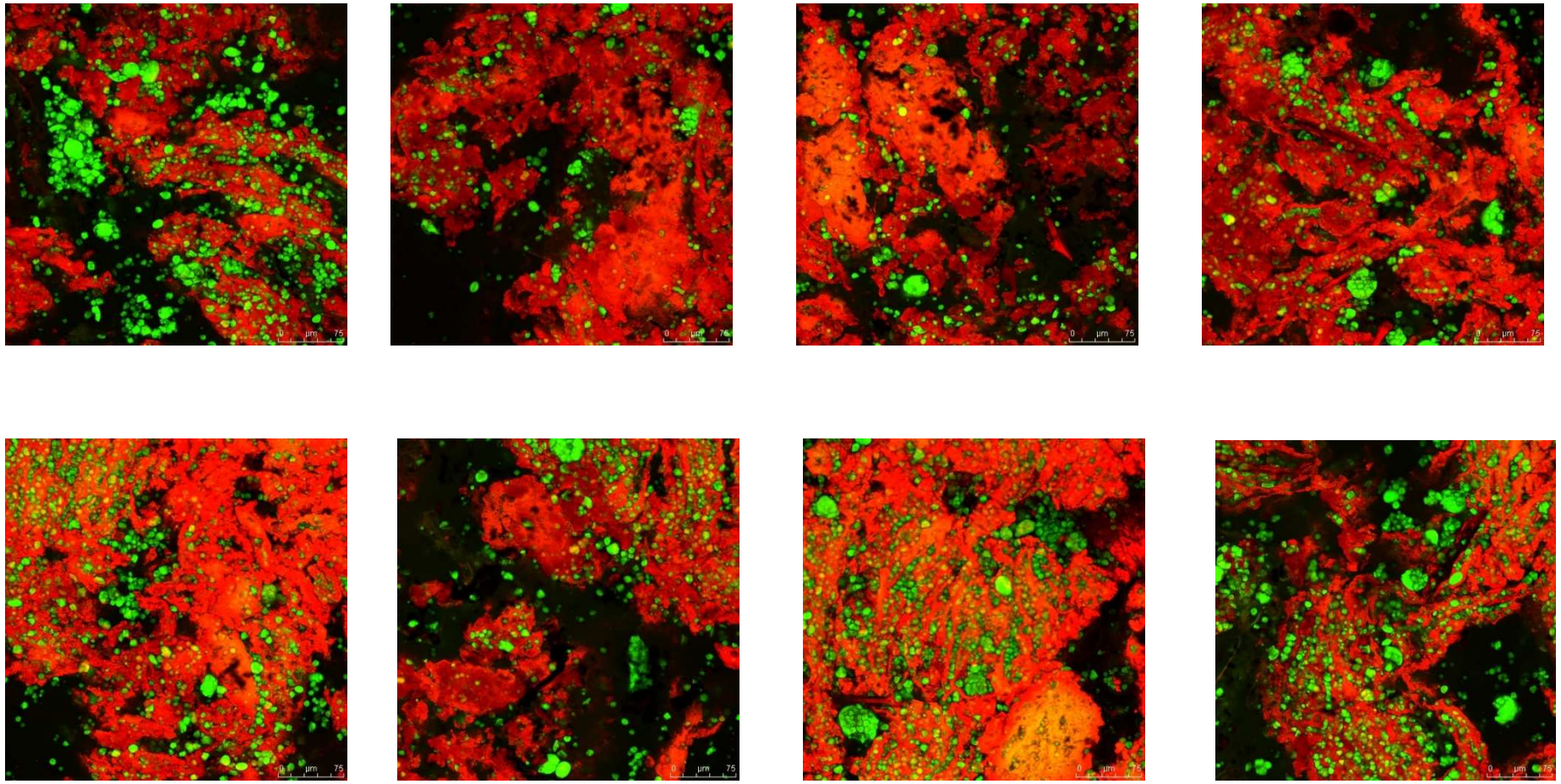


Figure C.3. Sample 16 Confocal Scanning Laser Microscope Images

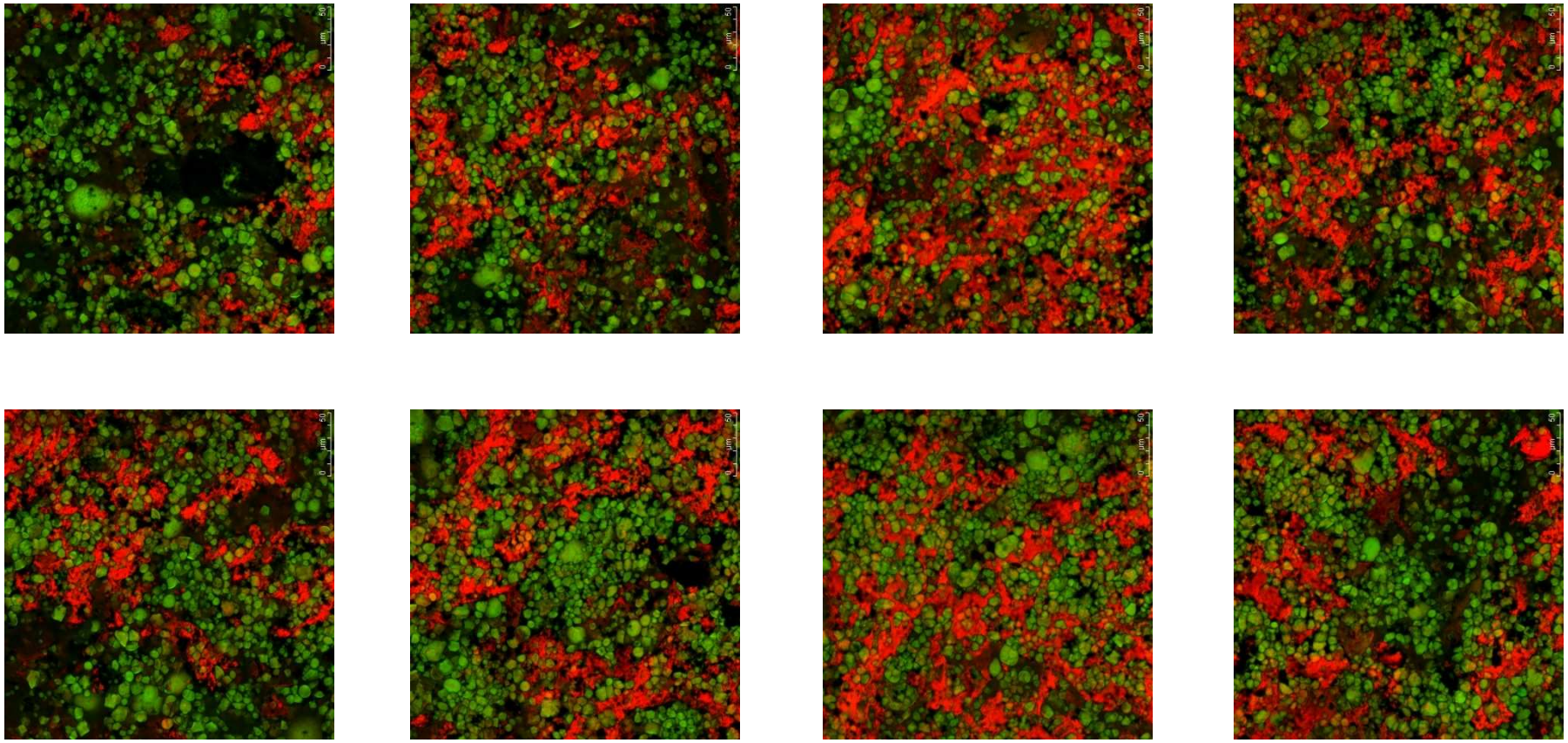
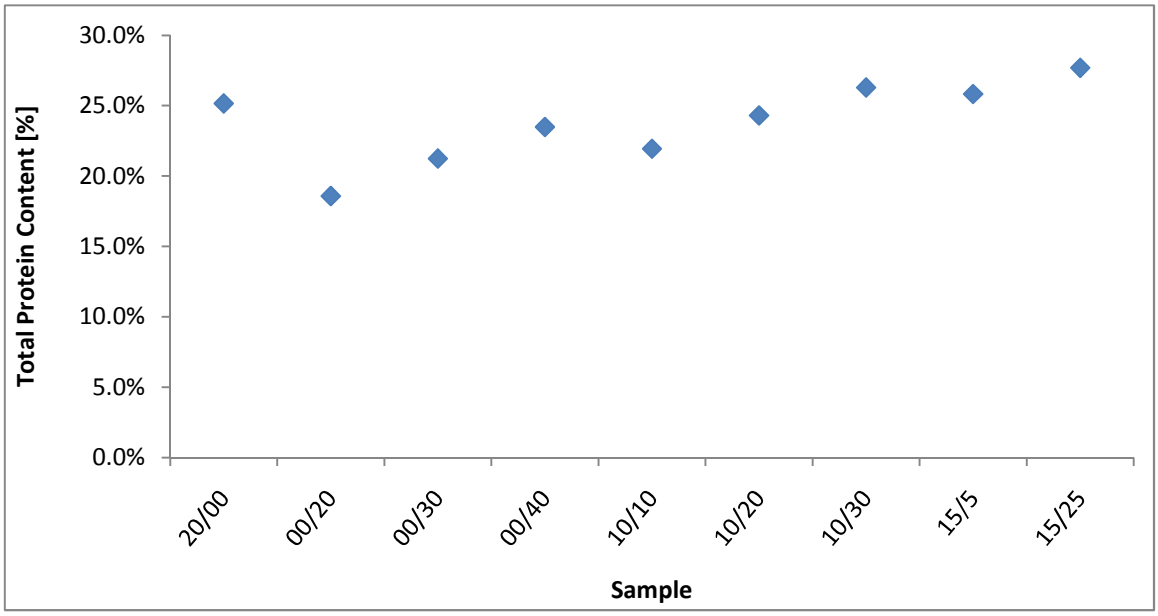


Figure C.4. Sample 20 Confocal Scanning Laser Microscope Images

15. Appendix D



Oat-Gluten Protein Functionality Supporting Data

Figure D.1. Total protein content of each dough sample.

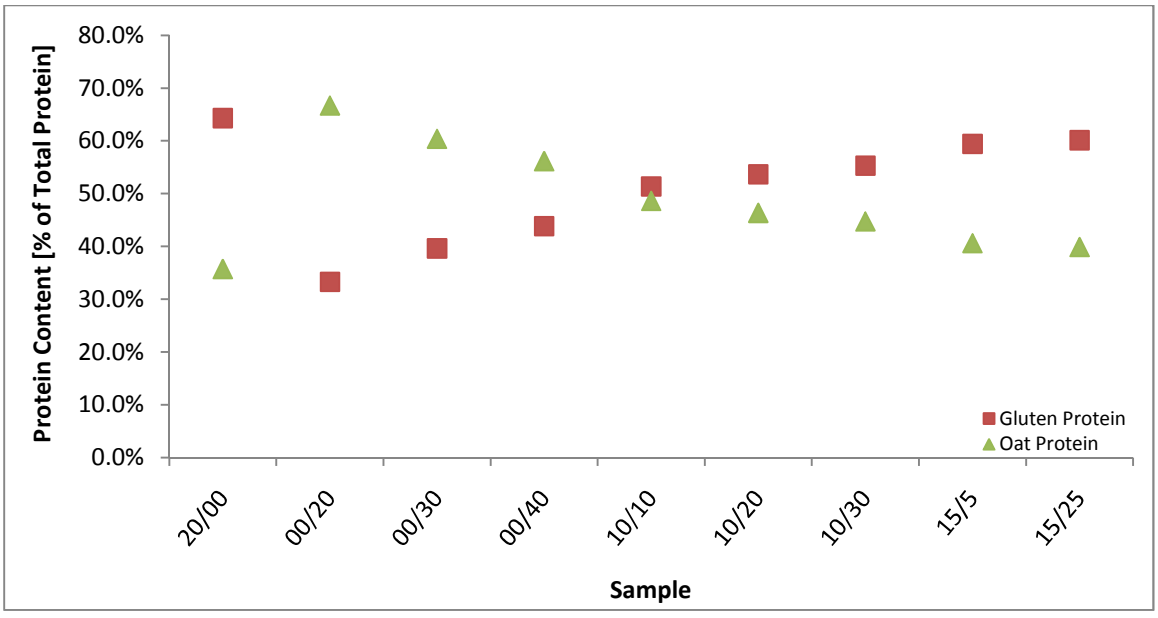


Figure D.2. Gluten and oat protein content of each dough sample, as a percentage of the total protein.

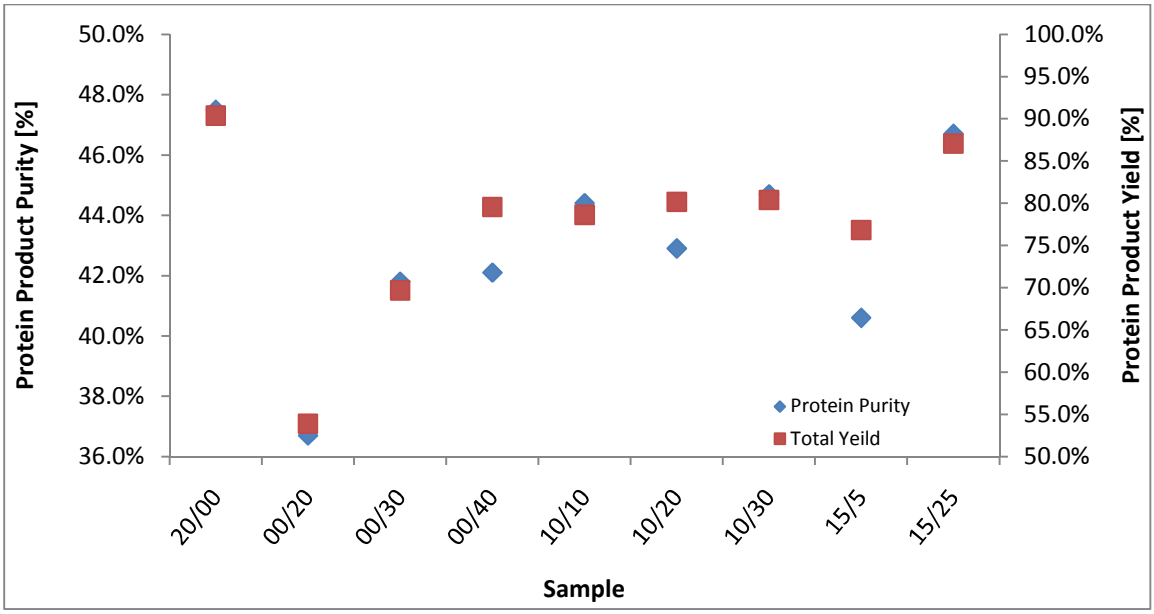


Figure D.3. Protein product purity and protein product yield for each sample

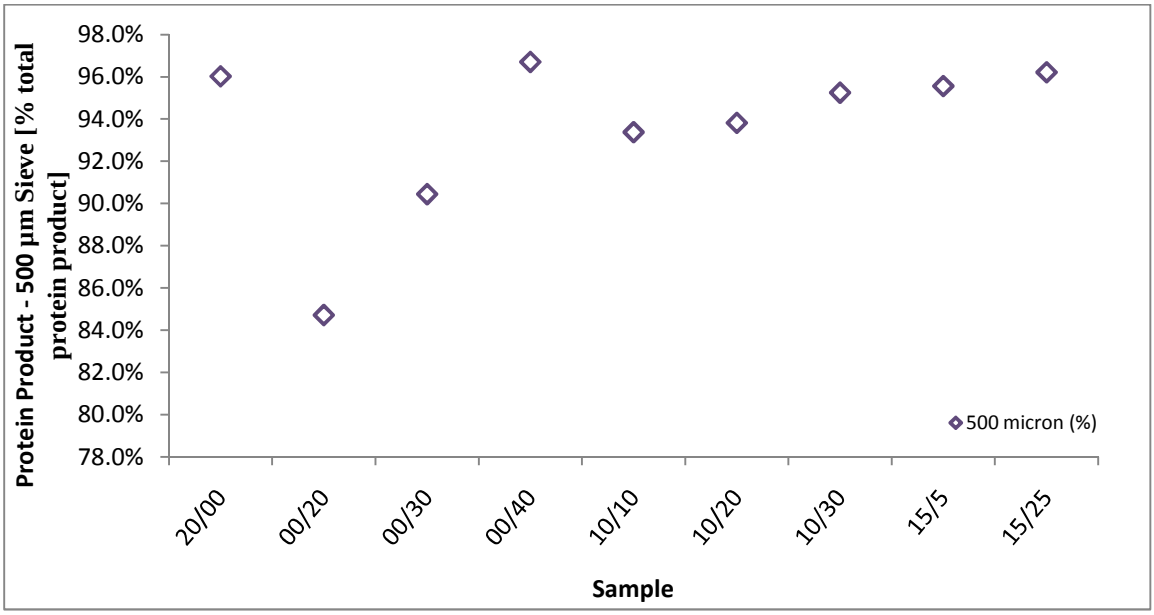


Figure D.4. Protein product recovered by the 500 µm sieve as a percentage of the total protein product recovered, for each sample.

16. Appendix E

Protein Product Yield - 400 µm sieve and 125 µm sieve

Small Pilot Scale

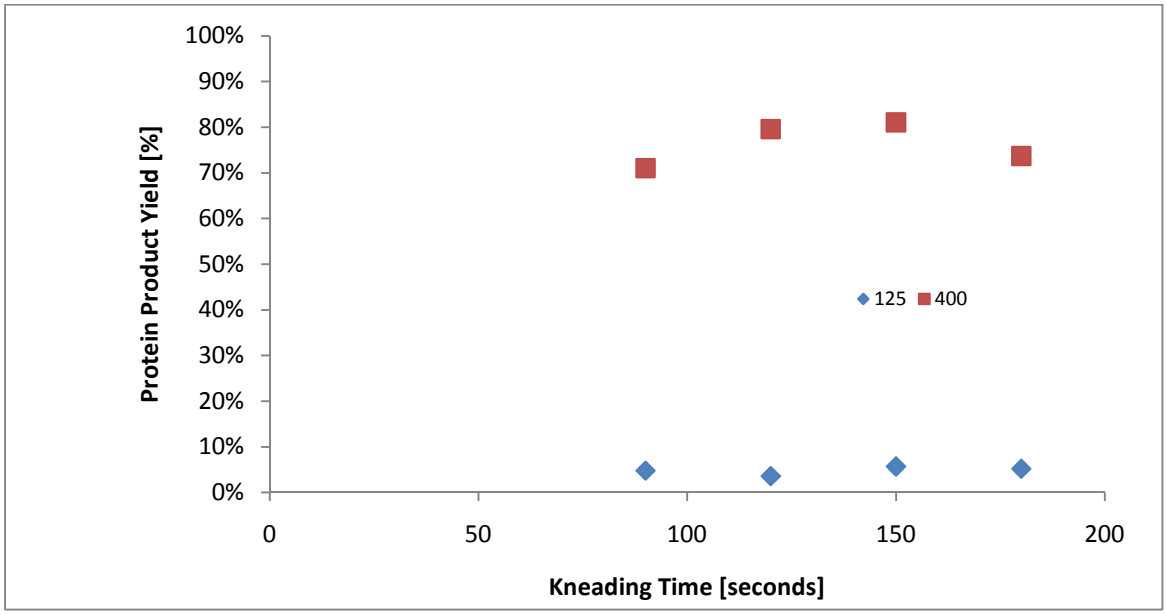


Figure E.1. The effect of oat-gluten dough kneading time on the protein product yield recovered on the 400 µm and 125 µm sieves

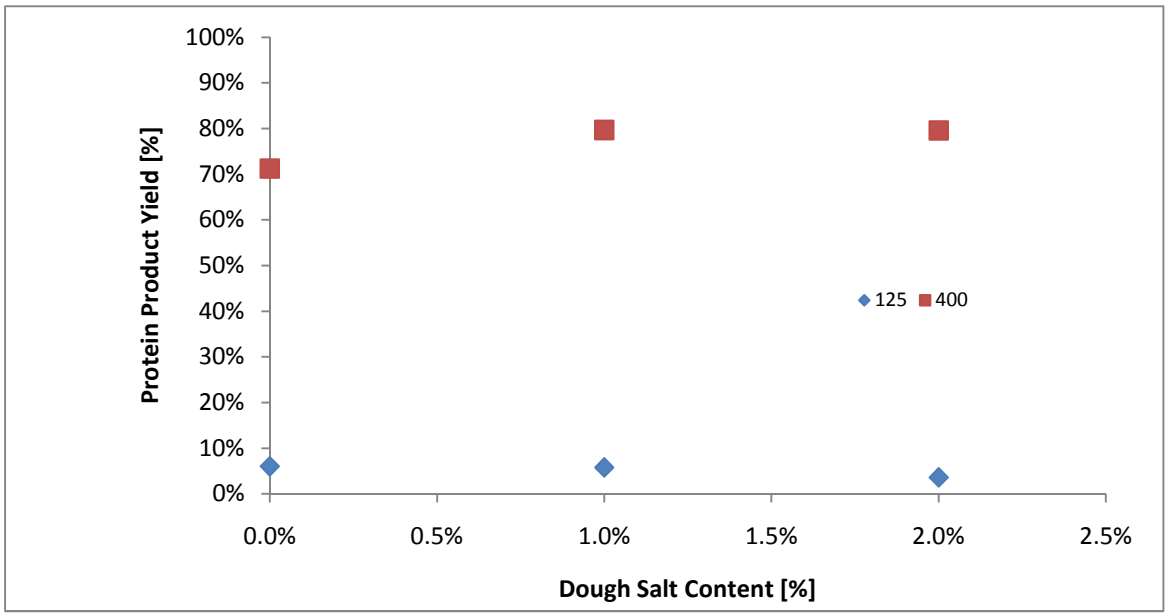


Figure E.2. The effect of the sodium chloride content of the oat-gluten dough on the protein product yield recovered on the 400 µm and 125 µm sieves

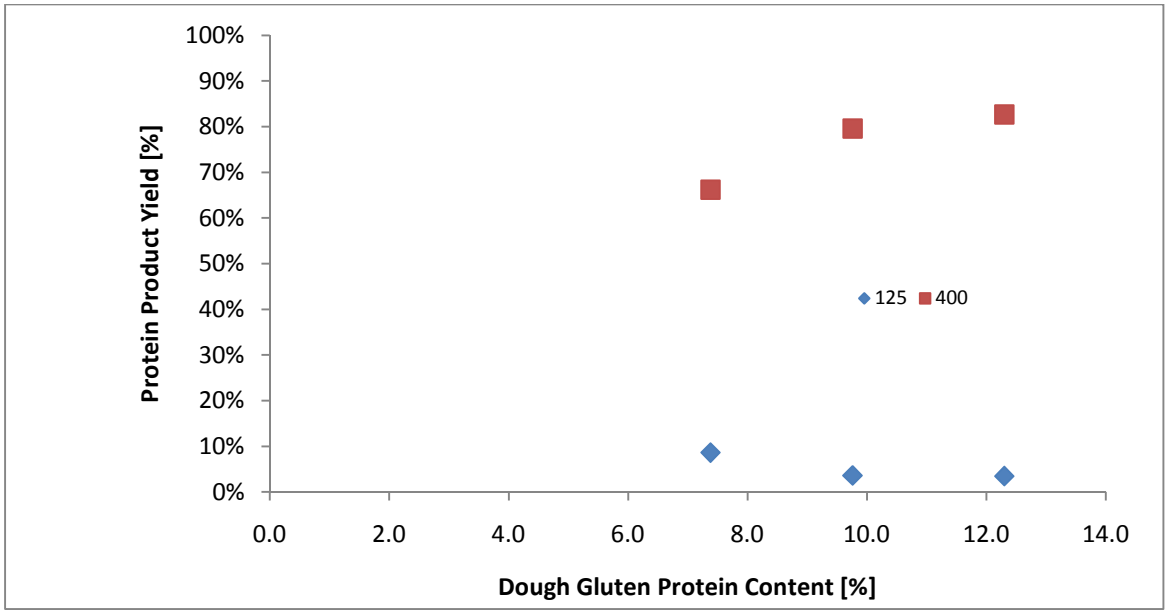


Figure E.3. The effect of gluten protein content of the oat-gluten dough on the on the protein product yield recovered on the 400 µm and 125 µm sieves

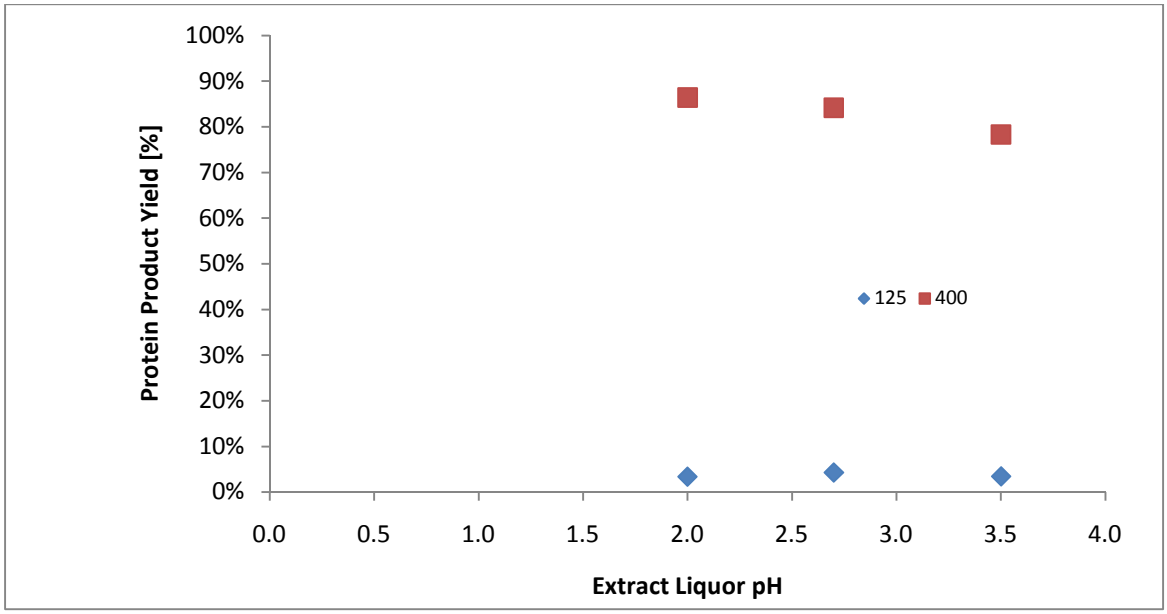


Figure E.4. The effect of the extract liquor pH on the protein product yield recovered on the 400 µm and 125 µm sieves

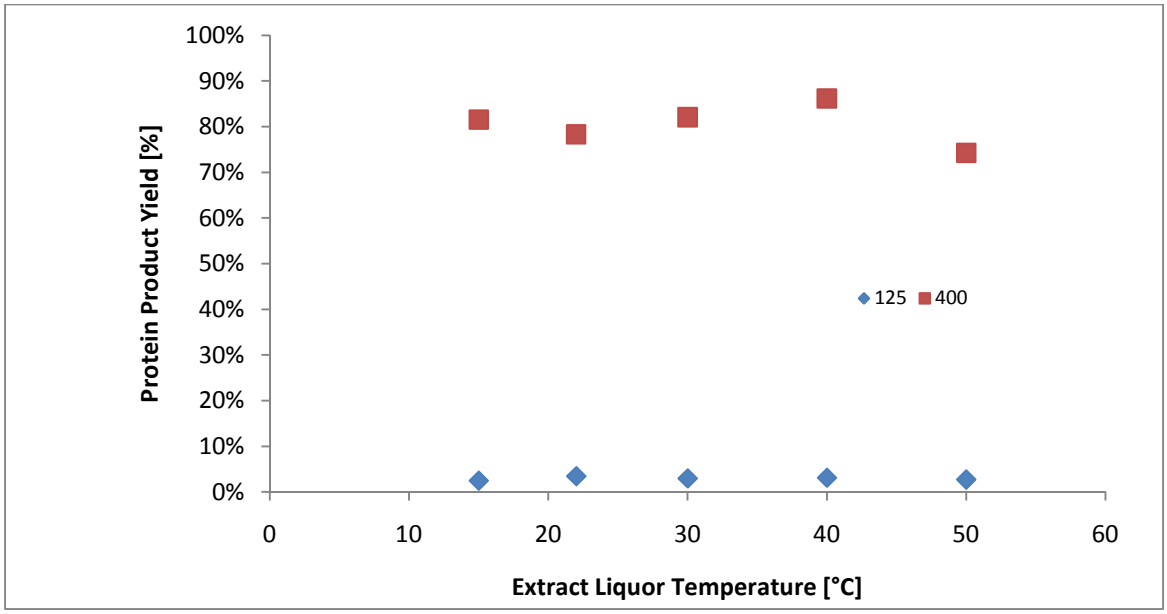


Figure E.5. The effect of the extract liquor temperature on the protein product yield recovered on the 400 µm and 125 µm sieves

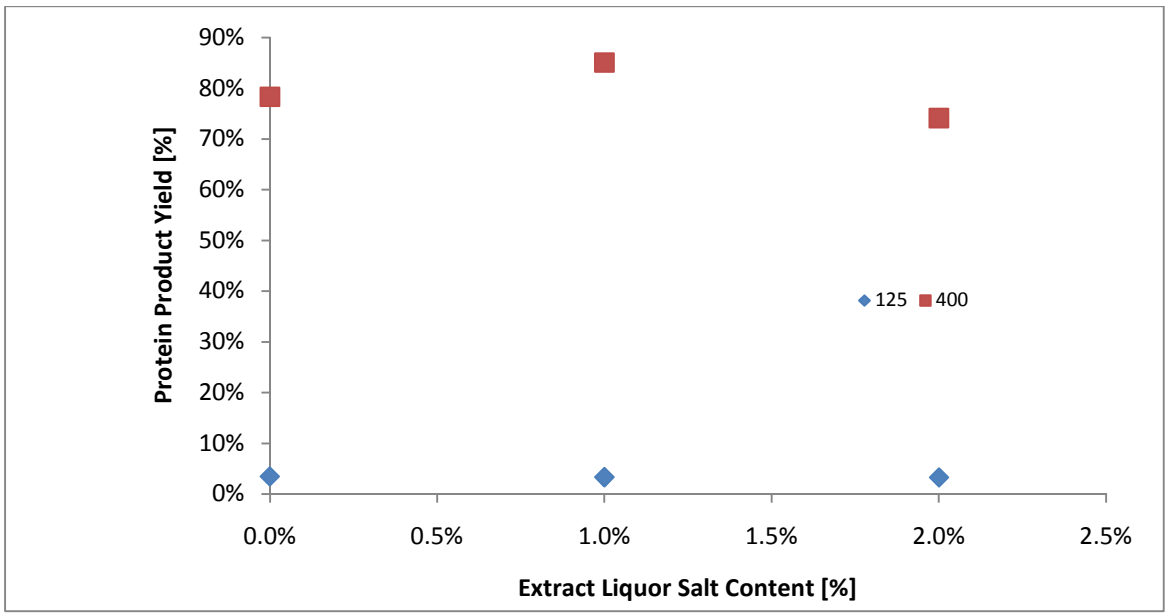


Figure E.6. The effect of the sodium chloride content of the extract liquor on the protein product yield recovered on the 400 µm and 125 µm sieves

17. Appendix F

Al-Hakkak Process Recommended Operating Conditions

This research has identified recommended operating conditions for several key processing parameters. For some processing parameters optimal operating points have been identified. For other processing parameters the benefits and penalties of varying the operating conditions have been established. Recommended operating conditions for dough processes are presented in **Table 17.1**, for extraction and purification in **Table 17.2**, and for drying in **Table 17.3**.

Table 17.1. Recommended Operating Conditions For Dough Manufacture

Parameter	**Operating condition description**
Oat flour	48% of dough mass.
Gluten flour	12% of dough mass. Oat-gluten protein product from the Al-Hakkak Process can be substituted for gluten flour. The addition of oat-gluten protein should be calculated based on the gluten protein content of the oat-gluten protein product.
Sodium chloride	0.01% sodium chloride concentration by mass in the dough.
Water	40% of dough mass.
Kneading water temperature	30 °C.
Kneading time	The kneading time must be optimised depending on the scale and type of mixer used. For example: • Larger pilot scale: 10 minutes using the 5kg Hobart mixer. • Small pilot scale: 150 seconds using the Farinograph mixer.
Resting time	Minimum resting time of greater than the characteristic resting time (approximately 14 minutes). Very long resting times (greater than 90 minutes) are not recommended as it has not been established if changes in the oat-gluten dough over a long period (such as microbial or enzymatic activity).

Table 17.2. Recommended Operating Conditions for Extraction and Purification

Parameter	Operating condition description
Extract water volume	40 g extract water for every 10 g oat-gluten dough.
Extraction time	The extraction time should be optimised depending on the scale of mixer used. For example between 40 minutes and 60 minutes at 75 rpm in the 500ml AgResearch designed vessel.
Extraction temperature	40 °C.
Extraction pH	Natural uncontrolled pH (approximately pH 4) is recommended.
Sodium chloride content of extract liquor	1% sodium chloride.
Protein and starch separation	Sieving using a 400 μm sieve.
Purification water volume	40 g purification water for every 10 g oat-gluten dough.
Extraction temperature	40 °C.
Extraction time	The purification time should be optimised depending on the scale of mixer used. For example between 40 minutes and 60 minutes at 75 rpm in the 500ml AgResearch designed vessel.

Table 17.3. Recommended Operating Conditions for Drying

Parameter	Operating condition description
Starch slurry pH	Acidification of the starch slurry to enhance settling is not recommended at this altered the structural characteristics of spray dried starch granule agglomerates.
Starch purification	Starch purification should be carried out if smaller agglomerates and individual starch granules are desired.
Drying	Spray drying is recommended. • Inlet air temperature ~ 140 °C • Outlet air temperature ~ 80 °C.